SUJMS
Sana'a University Journal of Medical Sciences

Original Article

Stem cell, types, and its role in the production of functional cells: Mini review

Lutfi Al-maktri[1] , Faisal Ali[2] ,and Mohammed AK. Al-Nuzaily [3]

1Haematology Department, Faculty of Medicine and Health Sciences, Sana a University, Yemen.

2Department of Nutrition and Dietetics, Metabolism and Genomics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

3Kuwait University Hospital, Haematology Department, Faculty of Medicine and Health Sciences, Sana a University, Yemen.

Abstract

It is currently well known that stem cells are mother cells that possess the capacity to become any type of cell in the body. Stem cells are cells without specific structure and characterized by their ability to self-renew or multiply while maintaining the potential to develop into other types of cells. Stem cells can normally become cells of the blood, heart, bones, skin, muscles or brain. Although, there are different sources of stem cells, all types of stem cells have the same capacity to develop into multiple types of cells. Stem cells are generally described as unspecialized cells with unlimited proliferation capacity that can divide (through mitosis) to produce more stem cells. Several types of adult stem cells have been characterized and can be cultured in vitro, including neural stem cells, hematopoietic stem cells, mesenchymal stem cells and epithelial stem cells. They are valuable as research tools and might, in the future, be used to treat a wide range of diseases such as Parkinson's disease, diabetes, heart disease and many other diseases. Currently, two types of stem cells have been identified based on their origin, namely embryonic stem cells and adult stem cells. Bone marrow is a rich source of stem cells that can be used for research purposes and to treat some blood diseases and cancers.

Keywords: stem cells, stem cell types and biology, stem cell uses, molecular and cellular markers.

INTRODUCTION:

Stem cells

The discovery of stem cells is largely referred to Professor Alexander A. Maximow from 1922 to 1928. He was a Russian-American physician, embryologist and histologist. He developed his theory that all cells come from the same precursor cell, which he later calls as stem cells. He proposed the existence of hematopoietic stem cells and described his unitarian theory of hematopoiesis during that event. He was the first person to use the term "stem cells" for scientific and medical purposes. Later, other physicians and medical researchers such as Joseph Altman, Gopal Das, Andre Gernez, McCulloch and till discovered various characteristics of stem cells. They first found methods to isolate stem cells from mouse and then human embryos. Later, they grow the cells in the laboratory for infertility purposes through in vitro fertilization procedures (1).

Generally, stem cells are defined as unspecialized cells with unlimited proliferation capacity that can differentiate into various cell types with specific functions. Two types of stem cells have been identified based on their origin, namely a) embryonic stem cells (ES) or fetal stem cells and b) adult stem cells. ES cells are obtained from tissues of a developing human fetus and these cells have some characteristics of the tissues they are taken from, while adult stems cells are obtained from some tissues of the adult body, for example, bone marrow. Bone marrow is a rich source of stem cells that can be used to treat some blood diseases and cancers (2, 3).

Fetal stem cells are present during the early stages of embryonic development in the inner cell mass of the

blastocyst. These possess the capacity to divide for long periods and retain their ability to make all cell types within the organism in the developing mouse (4, 3). Fetal stem cells are therefore called pluripotent. Adult stem cells are referred to as multipotent, indicating that they have a less wide-ranging potential than ES cells, and that their differentiation capacity is restricted to particular cell types in the body. Adult stem cells have been found in various tissues throughout the body where they reside in a specific micro-environment, also referred to as the stem cell niche, which is formed by differentiated somatic cells and extracellular matrix. For proper development and tissue homeostasis, stem cells receive multiple signals from their niche, including secreted cytokines and growth factors, as well as signals mediated by cell-cell interactions and interactions with extracellular matrix which control the processes of stem cell inactivity, self-renewal and differentiation (5, 6). Stem cells are not only required for development and organogenesis, but also for tissue maintenance and tissue repair (7,8). For these processes, the stem cell compartment is essential to give the full range of differentiated cells while also maintaining a pool of undifferentiated cells. This is accomplished by a characteristic property of stem cells, namely their ability to self-renew. This implies that stem cells can proliferate while maintaining their pluripotent or multipotent character. Self-renewal can also occur by so-called asymmetrical cell division, which implies that upon division one daughter cell becomes a committed progenitor cell that subsequently undergoes further differentiation, while the other daughter cell remains a stem cell with identical characteristics as the mother cell, thereby keeping the size of the stem cell pool intact (6).

Stem cell biology

Stem cell biology is of great clinical and scientific interest. Understanding stem cell development will provide molecular insight in such processes as early embryogenesis, but also in tumour formation and e.g. autoimmune diseases. Both embryonic and adult stem cells have been shown to be able to differentiate into specialized cell types and contribute to the restoration of damaged tissue after transplantation into rodents (9). Stem cell biology is therefore of particular interest for its possible applications in regenerative medicine whereby damaged or dysfunctional cells are replenished in such cases as Parkinson's disease, heart failure, diabetes mellitus or skin burns. Several types of adult stem cells have been characterized and can be cultured in vitro, including neural stem cells, hematopoietic stem cells, mesenchymal stem cells and epithelial stem cells (10). Mesenchymal stem cells have already been applied in bone tissue engineering, by growing them on three-dimensional scaffolds that are subsequently implanted in vivo to produce new bone. In addition to optimization of their use in bone tissue engineering, understanding mesenchymal stem cell behavior can be of great relevance in order to understand and intervene with bone diseases such as osteoporosis. Of essential importance in understanding the mechanisms controlling stem cell differentiation, is to find answers to the following questions: 1) what are the extrinsic and intrinsic triggers that prevent or induce stem cells to differentiate into a specialized cell lineage, 2) what are the differentiation pathways induced and by which markers can such differentiated cells be characterized, 3) what mechanisms control the process of self-renewal? Solving these issues is also of clinical importance since development of stem cell culture techniques will eventually provide opportunities for controlling stem cell behaviourior ex vivo. The challenge will be to employ their self-renewal capacity to expand stem cells in culture in order to generate sufficient amounts of cells, and subsequently to trigger the cells to differentiate into a specific cell type. Transplantation into the body of such in vitro expanded and differentiated cells has promising potentials for replacing cells and tissue affected by degenerative disorders (11-13).

Stem cell proliferation and differentiation

The pluripotentiality of embryonic stem cells and the multipotentiality of adult stem cells implies that intricate stimulatory and regulatory mechanisms determine the irreversible commitment of such cells to acquire a particular specialized function. Although the lineage commitment and further differentiation of embryonic and adult stem cells may be diverse and cell type specific at the molecular level, some general concepts apply in the development of stem cells. Stem cells produce intermediate cell types with restricted differentiation capacity, called progenitors, which can be formed during asymmetric self renewal. Subsequently, progenitor cells can give rise to mature cell types. In comparison with their parent stem cells, progenitor cells are committed to a limited number of cell types generally within a single lineage. Furthermore, progenitor cells still have the ability to proliferate, but unlike stem cells they cannot self-renew. Stem cell differentiation has been considered to be hierarchical in nature, such that the generation of specialized cell types occurs stepwise through defined intermediate stages.

Hematopoietic stem cell differentiation

The best-described model of adult stem cell differentiation is that of the hematopoietic stem cell, from which all cells of the blood and immune system is formed. The model presented in (Fig.1) is illustrative for lineage commitment and further differentiation of adult stem cells. As a first event, hematopoietic stem cells can form two distinct progenitors, namely a myeloid and a lymphoid progenitor. The latter progenitor generates mature immune cells such as B lymphocytes, T lymphocytes and natural killer cells, while the myeloid progenitor differentiates into erythrocytes, platelets, neutrophils, macrophages and monocytes. According to this model, stem cells generate restricted progenitor cells that become gradually more specialized by losing their ability to differentiate along alternative pathways. Similar hierarchical models have been described for differentiation of other adult stem cells, including intestinal stem cells, epidermal stem cells and neural stem cells and, mesenchymal stem cells (15-17). Cytokines, growth factors (GF), and hormones are chemical messengers that mediate intercellular communication and stimulate cellular growth, proliferation and cellular differentiation of stem cells as shown in Figure 1. These factors and their receptors play an important role in the regulation of stem cell differentiation into cell of interest. By adding BMP, bone morphogenetic protein; EGF, epidermal growth factor; FGF, fibroblast growth factor; GM-CSF, granulocyte macrophage-colony stimulating factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor into specific media, stem cells were directed toward becoming early and mature cells in vitro . For example, rat bone marrow mesenchymal stem cells (BMSCs) could differentiate into hepatocytes in the differentiation media including hepatocyte growth factor (HGF) or beta-nerve growth factor (beta-NGF) and to find a new source for therapies of hepatic diseases. In the same context, human BMSCs differentiate into neural cells (NCs) in the presence of human epidermal growth factor (hEGF) and bovine fibroblast growth factor (bFGF) and neurobasal media media plus B27. When BMSCs were cultured with hEGF and bFGF, RNA expression of neuronal specific markers Nestin, MAP-2, and tyrosine hydroxylase (TH) were observed (17,18).

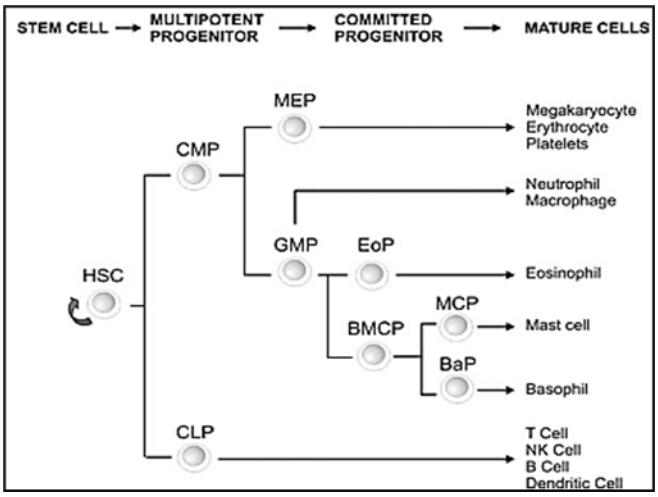

Figure1. Diagram showing the hierarchical model of hematopoietic stem cell differentiation. Hematopoietic stem cells (HSC) differentiate into specialized mature cells via progenitor cells. HSC can form two types of progenitor cells, namely a common myeloid progenitor (CMP) and a common lymphoid progenitor (CLP), which are restricted in their differentiation capacity relative to the parent HSC. The CLP can differentiate into cells of the immune system, while in its turn the CMP gives rise to progenitors that are committed to a limited number of cell types. According to this hierarchical model, progenitor cells gradually lose their ability to differentiate along alternative pathways. Abbreviations: MEP, megakaryocyte /erythrocyte progenitor; GMP, granulocyte/monocyte progenitor; EoP, eosinophyl progenitor; BMCP, basophil/mast cell progenitor; MCP, mast cell progenitor; BaP, basophil progenitor. Adapted from Bart vaes, thesis, 2007, ISBN: 978-90-9021853-3.

Changes of MSC in bone marrow of ageing

Age-related changes in the rate of bone formation are accompanied by a decrease in the number of bone-forming osteoblasts and an increase in the number of fat-storing adipocytes in the bone marrow of aging and osteoporosis persons, since the balance between osteogenesis and adipogenesis in bone marrow is gradually shifted in favor of adipogenesis (19,20). Moreover, Elena et al., (21) reported that aging causes a decrease in the commitment of marrow MSC to osteoblast lineage and an increase in the commitment to the adipocyte lineage in vitro.

Indeed, an increase in marrow adipocytes is associated with osteoporosis and age-related osteopenia, where in humans up to 90% of the marrow cavity is occupied by adipocytes resulting in the appearance of fatty marrow by the third decade of life. Thus, there is a clinical correlation between the appearance of bone marrow fat and reduced bone forming capacity (22 ,23). Furthermore, the reciprocal relationship between osteogenesis and adipogenesis, in combination with the increased adipogenesis during aging and osteoporosis, opens opportunities to decrease adipocyte differentiation, accompanied by an increase of osteoblast formation providing a therapeutic target to prevent further increases in adipocytes formation. (24,25).

Embryonic Mesenchymal stem cell differentiation

Mesenchymal stem cells (MSC) are multipotent adult stem cells of mesodermal origin. These stem cells are able to differentiate into a number of different cell types including osteoblasts (bone cells), adipocytes (fat cells), condrocytes (cartilage cells) and myoblasts (muscle cells). Cultured stem cells provide model systems by which the mechanisms that recapitulate lineage commitment and cellular differentiation during development can be studied. Differentiation of embryonic and adult stem cells into mature cell types has been accomplished successfully upon in vitro culture (26) and both ES cells and MSC can undergo osteoblast differentiation in vitro. Mammalian ES can be maintained as long-term self-renewing cell lines in culture and have been isolated from blastocyst stage embryos of several species, including mouse , human (27), monkey (28) and pig (29). When grown in suspension culture, ES form small aggregates of cells surrounded by an outer layer of visceral endoderm (30). Such aggregates have been termed embryoid bodies and are often used as model systems for studying differentiation and gene expression control during early development. Human ES can be induced to differentiate into osteoblasts in vitro (31, 32), but most studies have been carried out using murine ES cells. Dependent on culture conditions, such ES can give rise to cell populations from the mesenchymal lineage including skeletal muscle (33), adipocytes , chondrocytes (34) and osteoblasts (35,36). Differentiation of the established murine ES cell line D3 into osteoblasts can be induced by a combination of dexamethasone, ascorbic acid and β-glycerophosphate (dex/aa/β-glyc) (35), and is enhanced by vitamin D3 (36). Kawaguchi et al. showed that BMP4 induces osteoblast marker gene expression in another murine ES cell line, but additional dex/aa/β-glyc was necessary to induce calcium deposition. BMP4 and

BMP2 have also been shown to induce modest induction of Alcian blue-stained areas in ES cell cultures, which is indicative for chondrocyte differentiation (37). Induction of chondrogenesis by BMP was not observed in the experiment described by Kawaguchi et al., but could instead be induced by treatment with TGFB3 . A combination of BMP2 and TGFB1 has been shown to drive mouse ES towards both chondrogenesis and adipogenesis (34). As these examples illustrate, the outcome of ES differentiation in vitro appears highly dependent on the cell lines and/or culture conditions used. However, the distinct effects of various stimuli on these stem cells may also be indicative for the existence of different kinds of progenitors in culture.from the mesenchymal lineage including skeletal muscle (33), adipocytes (34), chondrocytes (37, 34) and osteoblasts (35, 36). Differentiation of the established murine ES cell line D3 into osteoblasts can be induced by a combination of dexamethasone, ascorbic acid and β-glycerophosphate (dex/aa/β-glyc) (35), and is enhanced by vitamin D3(249). Kawaguchi et al. showed that BMP4 induces osteoblast marker gene expression in another murine ES cell line, but additional dex/aa/β-glyc was necessary to induce calcium deposition (24). BMP4 and BMP2 have also been shown to induce modest induction of Alcian blue-stained areas in ES cell Cultures, which is indicative of chondrocyte differentiation (37). Induction of chondrogenesis by BMP was not observed in the experiment described by Kawaguchi et al., but could instead be induced by treatment with TGFB3(123). A combination of BMP2 and TGFB1 has been shown to drive mouse ES towards both chondrogenesis and adipogenesis (34). As these examples illustrate, the outcome of ES differentiation in vitro appears highly dependent on the cell lines and/or culture conditions used. However, the distinct effects of various stimuli on these stem cells may also be indicative of the existence of different kinds of progenitors in culture.

Adult MSC stem cells

The differentiation capacity of adult stem cells is limited in comparison with embryonic stem cells. Adult stem cells thus provide a less complicated model system to study differentiation pathways. However, the multipotential capacity of adult stem cells still allows for studies on lineage specification and stem cell self-renewal. MSC derived from bone marrow form the model system that is generally being used for studying the process of osteoblast differentiation. They form an adherent cell layer in tissue culture and have retained their multipotent capacity to form adipocytes, chondrocytes and osteoblasts (38, 39). Osteoblast differentiation of MSC can be induced in culture media containing dexamethasone, ascorbic acid and β-glycerophosphate (40). Since MSC mineralize their extracellular matrix, as measured by von Kossa staining, it appears that all stages of osteoblast differentiation are represented in this model system. Studies with clonal cultures of MSC have shown that single cells have the intrinsic ability to differentiate into osteoblasts, chondrocytes and adipocytes. This tripotentiality of MSC implies that these cells can well be used to investigate how differentiation along a particular pathway is accomplished. On the other hand, in some studies the majority of cells had lost this capacity and were only bi- or monopotential under the assay conditions used (41), possibly as a result of progression of the cells towards a specific differentiation pathway. On the other hand, optimal culture conditions still have to be established which drive a tripotential MSC exclusively into a single differentiation direction. As a consequence of such variation in lineage potential and inhomogeneous differentiation, differentiating MSC often reflect a mixture of phenotypes, which may be a drawback for their use in microarray experiments. A further disadvantage of the use of such cells is that the amount of MSC that can be obtained from human bone marrow aspirates is limited while in addition MSC have a limited proliferative capacity which is (42, 41) accompanied with progressive shortening of telomeres in comparison with ES cells (42). Although their bone

forming capacity does not appear to be affected during this process of replicative senescence (40), MSC lose their multilineage differentiation capacity upon prolonged ex vivo expansion (43). Therefore, new batches of MSC need to be isolated frequently from donors. It is therefore a challenge to identify factors that promote self-renewal in order to prolong culture periods without losing differentiation capacity.

Primary osteoblasts

When research interest is focused on the differentiation and maturation of osteoblasts rather than on lineage commitment, primary cultures of preosteoblastic cells can be used as a model system. Primary osteoblasts are usually obtained by means of proteolytical release from neonatal rat or mouse calvaria, since this tissue can easily be dissected. Calvaria-derived cells provide a physiologically relevant model system for studying osteoblast differentiation since these cells are able to form bone-like nodules in vitro upon treatment with ascorbic acid and dexamethasone. These nodules are formed by proliferation and differentiation of osteoprogenitor cells that constitute less than 1% of the total number of cells in the isolated population (44). Two distinct populations of such osteoprogenitors are present in the primary culture; one population is formed by immature Akp2-negative cells which require dexamethasone for differentiation, and the other by more mature Akp2-positive cells that differentiate in the absence of dexamethasone (45). These cultures thus represent a heterogeneous mixture of osteoblast progenitors at different developmental stages. The majority of these progenitors is restricted to the osteoblast lineage and thus unable to differentiate into adipocytes (46). However, as a population, calvaria-derived primary cultures do express adipogenic markers under osteogenic differentiation conditions (47), likely due to the presence of osteo-adipoprogenitors and committed adipocyte progenitors in the cell culture. Calvaria-derived osteoblasts are thus a valid model system to study bone formation, but the heterogeneity of the culture and the restricted differentiation capacity of the cells is a limitation for the investigation of mechanisms that underlie osteoblast commitment in relation to other mesenchymal lineages.

Model system to study MSC differentiation

Various model systems have been employed to study osteoblast differentiation in vitro, including cultured stem cells, primary cells and cell lines. The ideal model system to study (stem) cell commitment and further progression along the osteoblast lineage would have to represent all stages of osteoblastogenesis from multipotency to lineage commitment and matrix mineralization. In addition, the model system must preferably undergo homogeneous differentiation with respect to time and induced cell type, in order to achieve the highest specificity of gene expression in a microarray-based screening. Due to their limited proliferation capacity, MSC and primary cultures need to be isolated freshly, thereby introducing possible variations between experiments as a result of cell isolations from different donors. Clonal cell lines have the advantage that they provide homogenous populations that can often be maintained in culture for many passages. A well-established model system to study osteoblast differentiation and maturation in culture is formed by the murine cell line MC3T3, a spontaneously immortalized alkaline phosphatase-rich clone from a population of calvaria-derived cells (48). The cells behave as immature, committed osteoblasts that further upregulate alkaline phosphatase and osteocalcin protein levels by stimulation with bone morphogenetic proteins (BMPs), a group of growth factors ((BMP2 through BMP7) belong to the Transforming growth factor beta superfamily of proteins (49) and are able to form a mineralized matrix when cultured in the additional presence of ascorbic acid and β-glycerophosphate (49, 50). MC3T3 cells provide a mono-potential model system for osteoblast maturation and mineralization, but since these cells are already committed toward the osteoblast lineage, they give no information about the early events involved in cell type determination.

In order to study mechanisms of lineage specification, it is necessary to use cells with the flexibility to differentiate along multiple mesenchymal lineages. Examples of cell lines with this property are the adult mouse bone marrow-derived cell line ST2, and the fibroblastic cell line C3H10T1/2 which has been derived from early mouse embryos. ST2 cells differentiate into osteoblasts by treatment with BMPs (51) or ascorbic acid (52) and into adipocytes by treatment with insulin (53). C3H10T1/2 cells can be stimulated by various BMPs to differentiate into the three cell types osteoblasts, chondroblasts, and adipocytes, dependent on the applied BMP concentration (54, 55). This tripotentiality makes the C3H10T1/2 in principle suitable for investigating the early commitment of multipotent stem cells. However, for reasons that are not well understood, C3H10T1/2 cells show a heterogeneous response to BMPs, resulting in a mixture of osteoblasts, chondrocytes and adipocytes (54, 56). Using such a mixed population, it would be impossible to address changes in gene expression to a particular cell type.

Conclusion

Stem cells is a master regulator of the development of multicellular organisms and distinct regeneration processes throughout their lives, e.g., substituting diseased and damaged tissues. There are different types of stem cells based on the tissue origin and its function in terms of mature cells production. They are unspecialized precursor cells characterized by their abilities of self-renewal and pluripotency that means they can undergo numerous rounds of cell division maintaining the undifferentiated state and are able to develop into different types of cells such as skin, muscle or blood cells. Finally, controlling cell proliferation and differentiation requires additional basic research on the molecular and genetic signals that regulate cell division and specialization.

Acknowledgements.

We would like to thank our group members for their reviewing and helpful discussions during the preparation of this review and library in Universiti Putra Malaysia,Faculty of Medicine and Health sciences for assistance with collection of references.

References

1-http://www.news-medical.net/health/What-are-Stem-Cells.aspx. Accessed, 12 January, 2014.

2- Brook, F. A., and Gardner, R. L. (1999) The origin and efficient derivation of embryonic stem cells in the mouse. Proc Natl Acad Sci U S A 94:5709-12.

3- Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W., and Roder, J. C. (1993) Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. Proc Natl Acad Sci U S A 90:8424-8;.

4- Nagy, A., Gocza, E., Diaz, E. M., Prideaux, V. R., Ivanyi, E., Markkula, M., and Rossant, J. (1990) Embryonic stem cells alone are able to support fetal development in the mouse. Development 110:815-21

5.- Fuchs, E., Tumbar, T., and Guasch, G. (2004) Socializing with the neighbors: stem cells and their niche. Cell 116:769-78;

6. Watt, F. M., and Hogan, B. L. (2000) out of Eden: stem cells and their niches. Science 287:1427-30;.

7- Joseph, N. M., and Morrison, S. J. (2005) Toward an understanding of the physiological function of Mammalian stems cells. Dev Cell 9:173-83;

8- Kruger, G. M., and Morrison, S. J. (2002) Brain repair by endogenous progenitors. Cell 110:399-402.

9- Passier, R., and Mummery, C. (2003) Origin and use of embryonic and adult stem cells in differentiation and tissue repair. Cardiovasc Res 58:324-35.

10- Ramos, C. A., Venezia, T. A., Camargo, F. A., and Goodell, M. A. (2003) Techniques for the study of adult stem cells: be fruitful and multiply. Biotechniques 34:572-8, 580-4, 58691.

11- Bianco, P., and Robey, P. G. Stem cells in tissue engineering. (2001) Nature 414:11821.

12- Derubeis, A. R., and Cancedda, R. (2004) Bone marrow stromal cells (BMSCs) in bone engineering:

limitations and recent advances. Ann Biomed Eng 32:160-5.

13- Otto, W. R., and Rao, J. (2004) Tomorrow's skeleton staff: mesenchymal stem cells and the repair of bone and cartilage. Cell Prolif 37:97-110.

14- Radtke, F., and Clevers, H. (2005) Self-renewal and cancer of the gut: two sides of a coin. Science 307:1904-9.

15. Lavker, R. M., and Sun, T. T. (2000) Epidermal stem cells: properties, markers, and location. Proc Natl Acad Sci U S A 97:134735.

16- Wu, S., Wu, Y., and Capecchi, M. R. (2006) Motoneurons and oligodendrocytes are sequentially generated from neural stem cells but do not appear to share common lineage-restricted progenitors in vivo. Development 133:581-90.

17-Feng Z, Li C, Jiao S, Hu B, Zhao L. (2011) In vitro differentiation of rat bone marrow mesenchymal stem cells into hepatocytes. Hepato-gastroenterology journal. 58(112):2081-6.

18- Long X, Olszewski M, Huang W, Kletzel M. (2005) Neural cell differentiation in vitro from adult human bone marrow mesenchymal stem cells. Stem Cells Dev. 14(1):65-9.

19-Nuttall MA, Gimble JM. (2004) Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. Curr Opin pharmacol 4:290-294.

20-Verma S, Rajaratnam JH, Denton J, Hoyland JA and Byers RJ. (2002)"Adipocyte proportion of bone marrow is inversely related to bone formation in osteoporosis". J Clin Pathol 55(9):693-698.

21-Elena JM, Kui T, David A, Lipschitz and Beata LC. (2004) Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stoma/stem cells: the role of PPAR-gamma2 transcription facto and TGF-β/BMP signaling pathways. Aging cell 3:379-389.

22-Nuttle MA, Gimble JM. (2000) Is there a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis? .Bone 27 (2):177-184.

23- Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T and Kassem M. (2001) Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology 2(3): 165171.

24-Bianco P, Riminucci M, Gronthos S, Robey PG. (2001) Bone marrow stromal stem cells nature, biology, and potential applications. Stem cells 19:180-192.

25- Jiang Y, Jahagirder BN, Reinhardt RL, Schwartz RE, Knee CD, et al. (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41-49.

26- Keller, G. (2005) Embryonic stem cell differentiation: emergence of a new era in biology and medicine. Genes Dev 19:1129-55.

27-Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., and Jones, J. M. (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145-7.

28-Thomson, J. A., Kalishman, J., Golos, T. G., Durning, M., Harris, C. P., Becker, R. A., and Hearn, J. P. (1995) Isolation of a primate embryonic stem cell line. Proc Natl Acad Sci U S A 92:7844-8.

29- Li, M., Zhang, D., Hou, Y., Jiao, L., Zheng, X., and Wang, W. H. (2003) Isolation and culture of embryonic stem cells from porcine blastocysts. Mol Reprod Dev 65:42934.

30- Keller, G. M. (1995) In vitro differentiation of embryonic stem cells. Curr Opin Cell Biol 7:862-9.

31- Bielby, R. C., Boccaccini, A. R., Polak, J. M., and Buttery, L. D. (2004) In vitro differentiation and in vivo mineralization of osteogenic cells derived from human embryonic stem cells. Tissue Eng 10:1518-25.

32- Sottile, V., Thomson, A., and McWhir, J. (2003) In vitro osteogenic differentiation of human ES cells. Cloning Stem Cells 5:149-55.

33- Rohwedel, J., Maltsev, V., Bober, E., Arnold, H. H., Hescheler, J., and Wobus, A. M. (1994) Muscle cell differentiation of embryonic stem cells reflects myogenesis in vivo: developmentally regulated expression

of myogenic determination genes and functional expression of ionic currents. Dev Biol 164:87-101; .

34- Zur Nieden, N. I., Kempka, G., Rancourt, D. E., and Ahr, H. J. (2005) Induction of chondro-, osteo- and adipogenesis in embryonic stem cells by bone morphogenetic protein-2: effect of cofactors on differentiating lineages. BMC Dev Biol 5:1.

35-Buttery, L. D., Bourne, S., Xynos, J. D., Wood, H., Hughes, F. J., Hughes, S. P., Episkopou, V., and Polak, J. M. (2001) Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells. Tissue Eng 7:89-99.

36- zur Nieden, N. I., Kempka, G., and Ahr, H. J. (2003) In vitro differentiation of embryonic stem cells into mineralized osteoblasts. Differentiation 71:18-27.

37- Kramer, J., Hegert, C., Guan, K., Wobus, A. M., Muller, P. K., and Rohwedel, J. (2000) Embryonic stem cell-derived chondrogenic differentiation in vitro: activation by BMP-2 and BMP-4. Mech Dev 92:193-205.

38-Dennis, J. E., and Charbord, P. (2002) Origin and differentiation of human and murine stroma.Stem Cells 20:205-14.

39-Gregory, C. A., Prockop, D. J., and Spees, J. L. (2005) Non-hematopoietic bone marrow stem cells: molecular control of expansion and differentiation. Exp Cell Res 306:3305;.

40-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. J Cell Biochem 64:295-312.

41-Muraglia, A., Cancedda, R., and Quarto, R. (2000) Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 113 (Pt 7):1161-6; .

42-Banfi, A., Muraglia, A., Dozin, B., Mastrogiacomo, M., Cancedda, R., and Quarto,R. (2000) Proliferation kinetics and differentiation potential of ex vivo expanded human bonemarrow stromal cells: Implications for their use in cell therapy. Exp Hematol 28:707-15.

43- Muraglia, A., Cancedda, R., and Quarto, R. (2000) Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 113 (Pt 7):1161-6.

44-Bellows, C. G., and Aubin, J. E. (1989) Determination of numbers of osteoprogenitors present in isolated fetal rat calvaria cells in vitro. Dev Biol 133:8-13.

45-Turksen, K., and Aubin, J. E. (1991) Positive and negative immunoselection for enrichment of two classes of osteoprogenitor cells. J Cell Biol 114:373-84.

46- Bellows, C. G., and Heersche, J. N. (2001) The frequency of common progenitors for adipocytes and osteoblasts and of committed and restricted adipocyte and osteoblast progenitors in fetal rat calvaria cell populations. J Bone Miner Res 16:1983-93.

47-Garcia, T., Roman-Roman, S., Jackson, A., Theilhaber, J., Connolly, T., Spinella-Jaegle,S., Kawai, S., Courtois, B., Bushnell, S., Auberval, M., Call, K., and Baron, R. (2002) Behaviour of osteoblast, adipocyte, and myoblast markers in genome-wide expression analysis of mouse calvaria primary osteoblasts in vitro. Bone 31:205-11.

48-Chung, C. Y., Iida-Klein, A., Wyatt, L. E., Rudkin, G. H., Ishida, K., Yamaguchi, D. T., and Miller, T. A. (1999) Serial passage of MC3T3-E1 cells alters osteoblastic function and responsiveness to transforming growth factor-beta1 and bone morphogenetic protein-2. Biochem Biophys Res Commun 265:24651.

49-Franceschi, R. T., and Iyer, B. S. (1992) Relationship between collagen synthesis and expression of the osteoblast phenotype in MC3T3-E1 cells. J Bone Miner Res 7:235-46.

50- Franceschi, R. T., Iyer, B. S., and Cui, Y. (1994) Effects of ascorbic acid on collagen matrix formation and osteoblast differentiation in murine MC3T3-E1 cells. J Bone Miner Res 9:843-54.

51- Yamaguchi, A., Ishizuya, T., Kintou, N., Wada, Y., Katagiri, T., Wozney, J. M., Rosen, V., and Yoshiki, S. (1996) Effects of BMP-2, BMP-4, and BMP-6 on osteoblastic differentiation of bone marrow-derived stromal cell lines, ST2 and MC3T3-G2/PA6. Biochem Biophys Res Commun 220:366-71.

52-Otsuka, E., Yamaguchi, A., Hirose, S., and Hagiwara, H. (1999) Characterization of osteoblastic differentiation of stromal cell line ST2 that is induced by ascorbic acid. Am J Physiol 277:C132-8.

53- Ding, J., Nagai, K., and Woo, J. T. (2003) Insulin-dependent adipogenesis in stromal ST2 cells derived from murine bone marrow. Biosci Biotechnol Biochem 67:314-21.

54-Asahina, I., Sampath, T. K., and Hauschka, P. V. (1996) Human osteogenic protein-1 induces chondroblastic, osteoblastic, and/or adipocytic differentiation of clonal murine target cells. Exp Cell Res 222:38-47.

55-Wang, E. A., Israel, D. I., Kelly, S., and Luxenberg, D. P. (1993) Bone morphogenetic protein-2 causes commitment and differentiation in C3H10T1/2 and 3T3 cells. Growth Factors 9:57-71.

56-Date, T., Doiguchi, Y., Nobuta, M., and Shindo, H. (2004) Bone morphogenetic protein-2 induces differentiation of multipotent C3H10T1/2 cells into osteoblasts, chondrocytes, and adipocytes in vivo and in vitro. J Orthop Sci 9:503-8.