Journal of Cellular Biochemistry 104:620–628 (2008)

Distinct Osteoblastic Differentiation Potential of Murine Fetal Liver and Bone Marrow Stroma-Derived Mesenchymal Stem Cells

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Abstract Bone marrow-derived mesenchymal stem cells (MSC) are able to differentiate into osteoblasts under appropriate induction. Although MSC-derived osteoblasts are part of the hematopoietic niche, the nature of the stromal component in fetal liver remains elusive. Here, we determined the in vitro osteoblastic differentiation potential of murine clonal fetal liver-derived cells (AFT024, BFC012, 2012) in comparison with bone marrow-derived cell lines (BMC9, BMC10). Bone morphogenetic protein-2 (BMP2) increased alkaline phosphatase (ALP) activity, an early osteoblastic marker, in AFT024 and 2012 cells, whereas dexamethasone had little or no effect. BMP2, but not dexamethasone, increased ALP activity in BMC9 cells, and both inducers increased ALP activity in BMC10 cells. BMP2 increased ALP mRNA in AFT024, 2012 and BMC9 cells. By contrast, ALP was not detected in BMC10 and BFC012 cells. BMP2 and dexamethasone increased osteopontin and osteocalcin mRNA expression in 2012 cells. Furthermore, bone marrow-derived cells showed extensive matrix mineralization, whereas fetal liver-derived cell lines showed no or very limited matrix mineralization capacity. These results indicate that the osteoblast differentiation potential differs in bone marrow and fetal liver-derived cell lines, which may be due to a distinct developmental program or different microenvironment in the two hematopoietic sites. J. Cell. Biochem. 104: 620–628, 2008.

Key words: stromal cell; stem cell; osteoblast; fetal liver; bone marrow

The bone marrow cavity localized within bone trabeculae contains hematopoietic and non-hematopoietic microenvironmental cells. Microenvironmental cells include mature cells (vascular cells and adipocytes) and immature mesenchymal stem cells (MSC) [Owen, 1988; Caplan, 1991; Prockop, 1997; Bianco and Gehron Robey, 2000; Marie and Fromigue, 2006]. Bone marrow MSC are multipotential cells that can differentiate into several connective tissue cell lineages in vitro, each differentiation being dependent on local or hormonal factors [Ashton et al., 1985; Beresford et al., 1994; Fromigue et al., 1997]. MSC also provide the stromal component of the hematopoietic stem cell (HSC) niche [Muguruma et al., 2006], that is, the specific cellular and molecular microenvironment that allows HSCs to survive and to maintain the adequate balance between differentiation and self-renewal [Moore and Lemischka, 2006; Yin and Li, 2006]. Studies of clonal bone marrow MSC have indicated that a single MSC may have multipotential competence in vitro, including the potential to differentiate into osteoblasts [Dennis et al., 1999; Oyajobi et al., 1999; Pittenger et al., 1999; Muraglia et al., 2000]. Under appropriate culture conditions, clonal bone marrow stromal MSC reiterate the phenotypic sequence of events that characterize the differentiation and maturation of osteoblasts found in vivo [Bianco et al., 2001; Kassem, 2004; Oreffo et al., 2005; Marie and Fromigue, 2006]. The osteogenic differentiation of MSC is characterized by a stepwise sequence of phenotypic changes associated with cell proliferation and differentiation, and culminating in the production of

Grant sponsor: European Commission (Integrated Project #503161 Genostem); Grant sponsor: Etablissement Français du Sang (EFS) #2004-08 and EFS #2005-11; Grant sponsor: Inserm PNR (Pro-A); Grant sponsor: Fondation de l'Avenir.

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Received 27 September 2007; Accepted 30 October 2007
DOI 10.1002/jcb.21648

an extracellular matrix (ECM), which progressively mineralizes in vitro. Phenotypic marker genes that are timely expressed during osteogenic differentiation of MSC in vitro include alkaline phosphatase (ALP), type I collagen, osteopontin, bone sialoprotein, and osteocalcin [Malaval et al., 1994; Fromigue et al., 1997; Shui et al., 2003; Marom et al., 2005].

Several studies have shown that the osteogenic differentiation of bone marrow MSC requires induction by bone morphogenetic proteins (BMP) or dexamethasone [Ashton et al., 1985; Kamalia et al., 1992; Beresford et al., 1994; Cheng et al., 1994; Malaval et al., 1994; Rickard et al., 1996; Fromigue et al., 1997; Hanada et al., 1997; Jaiswal et al., 1997]. BMP2 acts by promoting the expression of osteoblastic markers and osteogenic differentiation [Rickard et al., 1994; Fromigue et al., 1998; Shui et al., 2003]. Multiple signaling pathways have been involved in BMP2-induced osteoblastic differentiation of MSC [Lee et al., 2000; Celil and Campbell, 2005]. Dexamethasone also induces osteoblastic differentiation by inducing multiple pathways in bone marrow MSC [Hanada et al., 1997; Qi et al., 2003; Osyczka and Leboy, 2005; Phillips et al., 2006; Ito et al., 2007]. Notably, a short and transient induction with dexamethasone is sufficient to induce osteoblastic lineage development in bone marrow-derived MSC [Fromigue et al., 1998].

Whereas bone marrow is the site of hematopoiesis after birth, liver is the other major hematopoietic site effective during fetal life [Dzierzak, 2001]. Fetal liver organization is strickingly different from that of bone marrow. Hematopoietic cells that consist mainly in erythroblasts are scattered among hepatoblasts. Although MSC have been detected [Campagnoli et al., 2001], whether they provide the stromal component of the HSC niche is still a matter of debate [Chagraoui et al., 2003]. Some fetal liver mesenchymal lines, whose differentiation potential and stromal capacity are similar to those of bone marrow MSC, may serve as model of fetal hematopoietic niche [Hackney et al., 2002; Chagraoui et al., 2003; Chateauvieux et al., 2007]. Such lines are rare, reflecting the clonal heterogeneity characteristic of mesenchymal lines whatever their origin [Zipori, 1989; Deryugina and Muller-Sieburg, 1993; Charbord et al., 2002].

In this study, we have investigated the potential to differentiate into osteoblasts of murine clonal mesenchymal lines derived from fetal liver [Hackney et al., 2002] versus bone marrow [Dennis et al., 1999]. We show here that the capacity to mineralize of bone marrow-derived lines is superior to that of fetal liver-derived lines, which is in agreement with the microenvironmental differences between the two sites of hematopoiesis. However, independent of their site of origin, mesenchymal lines show marked heterogeneity in their osteoblastic differentiation potential, which further emphasizes the heterogeneity of mesenchymal cell lines.

MATERIALS AND METHODS

Cell Lines

Liver-derived stromal cell lines were isolated from 14.5 dpc fetal liver lines (Lines AFT024, 2012 and BFC012 kindly provided by Dr. K. Moore (Department of Molecular Biology, Princeton University, NJ) [Thiemann et al., 1998]. Bone marrow-derived lines (BMC9, BMC10) were kindly provided by Dr. J.E. Dennis (Case Western Reserve University, Cleveland, OH) [Dennis et al., 1999]. The cell lines were immortalized using SV-40 large T antigen and their phenotype does not differ from primary cells [Charbord et al., 2002]. Cells were cultured in DMEM supplemented with L-Glutamin (292 mg/L), 10% heat-inactivated fetal calf serum (FCS), and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin). For the different experiments, cells were seeded at $1.25\,10^5$ cells/cm^2 and treated with the indicated compound the next day.

Induction of Osteoblast Differentiation

To induce osteogenic differentiation, the medium was supplemented with ascorbic acid (50 μg/ml) and inorganic phosphate (NaH_2PO_4; 3 mM) to induce collagenous matrix synthesis and mineralization [Quarles et al., 1992]. Dexamethasone (10^{-7}M; Sigma) or rhBMP2 (200 ng/ml; Genetics Institute, Cambridge, MA) were used as inducers of osteoblast differentiation [Fromigue et al., 1998].

Alkaline Phosphatase Activity

ALP activity was determined by a colorimetric assay for up to 14 days of the osteogenic differentiation process. Cells were lyzed and sonicated in ice cold H_2O. Lysates were centrifuged at 3,500 rpm for 15 min at 4°C and ALP

activity was evaluated using Sigma fast kit according to the manufacturer's recommendations (Sigma).

In vitro ECM Mineralization

After 7 or 14 days of culture, cells were fixed in 4% paraformaldehyde in PBS. Matrix mineralization was detected by alizarin red staining [Luppen et al., 2003] and microphotographed using an Olympus microsocope (Japan).

Semi-Quantitative RT-PCR Analysis

The expression of the early (ALP), intermediate (osteopontin; OP), and late (osteocalcin; OC) osteoblast markers were analyzed by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). After treatment with BMP2, dexamethasone or the solvent, the cells were washed with PBS and lysed with Trizol reagent (Eurobio, France) reagent according to the manufacturer's instructions. Three μg total RNA from each samples were reverse transcribed using MMLV reverse transcriptase and oligo-dT primers. Aliquots of cDNA samples were amplified using Taq polymerase (Eurobio) and specific primers as follows: sense 5′-AGGCAGGATTGACCACGG-3′ and antisense 5′-TGTAGTTCTGCTCATGGA-3′ for ALP; sense 5′-GAGCGGTGAGTCTAAGGAGT-3′ and antisense 5′-CTAAATGCAAAGTAAGGAAC-3′ for OP; sense 5′-AAGCAGGAGGGCAATAAGGT-3′ and antisense 5′-AGCTGCTGTGACATCCATAC-3′ for OC; sense 5′-AGCGATGATGAACCAGGTTA-3′ and antisense 5′-GTTGAGAGATCATCTCCACC-3′ for HPRT. Optimization of RT-PCR results was carried out by generating saturations curves of RT-PCR products of each gene against cycle number (0–35 cycles). We choose the same cycle number (30 cycles) for all genes, in which amplification was linear. The amplification products were then analyzed on 1.2% agarose gels. The signal for each gene was corrected for HPRT.

Statistical Analysis

The data were analyzed by two-factor analysis of variance (ANOVA) using the statistical package super-ANOVA (Macintosh, Abacus concepts, Inc., Berkeley, CA). A minimal level of $P < 0.05$ was considered significant.

RESULTS

Because ALP is an early osteoblastic marker which is upregulated during osteoblastic differentiation in MSC [Malaval et al., 1994], we first analyzed the changes in ALP activity in fetal liver-derived mesenchymal cells compared to the bone marrow-derived mesenchymal cells. As shown in Figure 1A, BMP2 rapidly (2 days) and markedly (up to 14-fold) increased ALP activity in BMC9 bone marrow-derived stromal cells, whereas dexamethasone had no effect. In contrast, both BMP2 and dexamethasone increased ALP activity by only from twofold to fourfold in BMC10 cells at late stage of cultures (Fig. 1A). These results indicate that ALP activity can be promoted by BMP2 or dexamethasone in murine clonal bone marrow-derived mesenchymal cells. We then investigated murine clonal fetal liver-derived mesenchymal cells. As shown in Figure 1B, BMP2 progressively and markedly (up to 10-fold) increased ALP activity in AFT024 cells. BMP2 slightly increased ALP activity in BFC012 and 2012 cells (Fig. 1B). Dexamethasone slightly increased (by twofold) ALP activity in ATF024, but not in BFC012 or 2012 cells (Fig. 1B). These results indicate that ALP activity is increased by BMP2 in the three murine fetal liver-derived stromal cells, albeit to highly variable degrees, the largest induction being observed in ATF024 cells, whereas dexamethasone had minor effect.

Induction of Osteoblastic Gene Expression

We then performed a semi-quantitative analysis of the expression of osteoblastic marker genes. According to the sequential expression during the osteogenic process, we analyzed changes in ALP, osteopontin, and osteocalcin, which are early, intermediate, and late osteoblastic markers, respectively. In BMC9 cells, ALP mRNA levels increased progressively with time in culture to reach a steady state by day 10 (Fig. 2A). Both BMP2 and dexamethasone increased by twofold to threefold ALP mRNA levels (Fig. 2A), which is consistent with the observed increase in ALP activity (Fig. 1A). By contrast, in BMC10 cells no detectable change in ALP mRNA level was found under BMP2 or dexamethasone stimulation (Fig. 2A), which is consistent with the small and late increase in ALP activity (Fig. 1A). Osteopontin mRNA levels remained unchanged under BMP2 or dexamethasone stimulation in both BMC9 and BMC10 cell lines (Fig. 2B). In BMC9 cells, osteocalcin mRNA levels were undetectable, even under stimulation with BMP2 or dexamethasone (Fig. 2C). By contrast, in

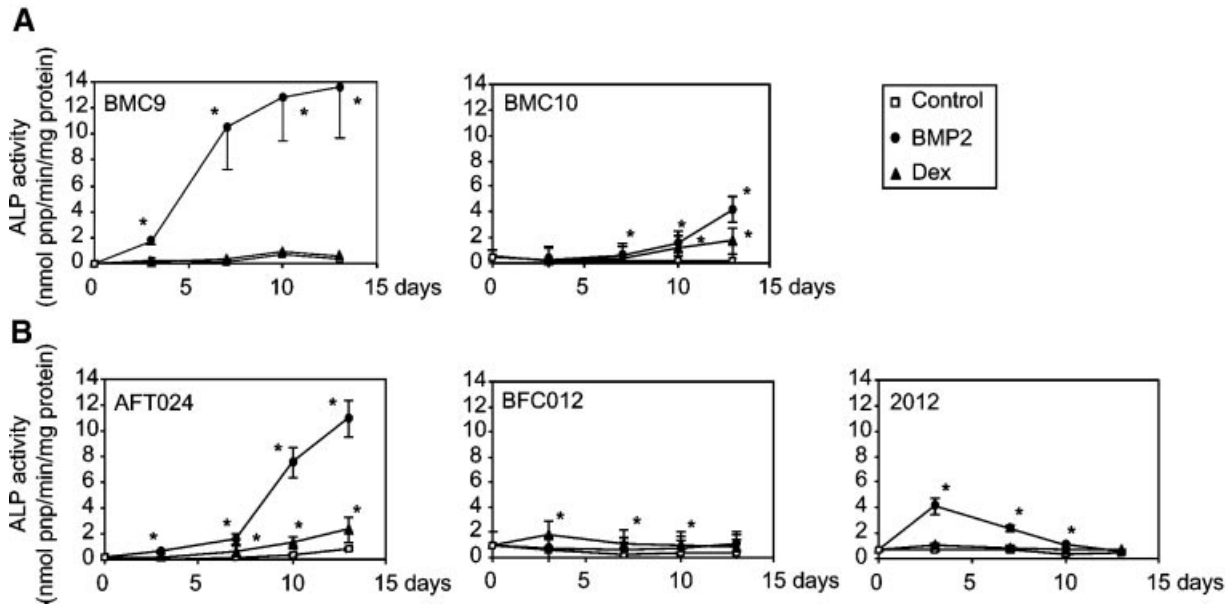

Fig. 1. Distinct induction of ALP activity in murine clonal fetal liver and bone marrow mesenchymal cells. Bone marrow-derived clonal (BMC9, BMC10) (**A**) and fetal liver cells (AFT024, BFC012, 2012) (**B**) were cultured for up to 14 days in the presence or absence of BMP2 (200 ng/ml) or dexamethasone (10^{-7}M), and alkaline phosphatase activity (ALP) was determined. *indicates a significant difference with untreated cells (Control). Note that ALP activity can be promoted by BMP2 or dexamethasone in murine clonal bone marrow and fetal liver stromal cells.

BMC10 cells OC mRNA levels were expressed and increased with time in culture; however, levels remained unchanged under BMP2 or dexamethasone stimulation (Fig. 2C). These results show that clonal bone marrow-derived BMC9 and BMC10 cells exhibit a different basal phenotypic pattern and a distinct pattern of osteoblastic marker gene expression in response to osteogenic stimuli.

We then investigated the pattern of osteoblastic marker gene expression in fetal liver-derived cells. In AFT024 cells, BMP2 increased ALP mRNA levels by twofold (Fig. 3A), which is consistent with the induction of ALP activity (Fig. 1B). In BFC012 cells, ALP mRNA was not expressed (Fig. 3A). In 2012 cells, BMP2 greatly increased ALP mRNA levels (Fig. 3A), confirming the increased ALP activity found in this cell line (Fig. 1B). We found OP gene was not expressed in AFT024 and BFC012 lines, even after BMP2 or dexamethasone stimulation. By contrast, in 2012 cells, both inducers increased OP mRNA levels, although with a variable time-course (Fig. 3B). In AFT024 cells, OC mRNA levels increased with time in culture regardless of BMP2 or dexamethasone stimulation (Fig. 3C). In BFC012 cells, OC mRNA was not expressed, even by day 14 (Fig. 3C). In contrast, both inducers increased OC mRNA levels by day 10 in 2012 cells (Fig. 3C). These results indicate that osteoblastic markers were not expressed in BFC012, in contrast to AFT024 or 2012 cells where a moderate or large increase was observed after BMP2 or dexamethasone stimulation, revealing different phenotypic characteristics in the three cell lines.

ECM Mineralization In Vitro

The differentiation of mesenchymal stromal cells into fully mature functional osteoblasts is characterized by the deposition and mineralization of an ECM. As shown in Figure 4, BMC9 and BMC10 bone marrow-derived mesenchymal cells cultured in basal conditions without inducers showed a remarkable capacity to synthesize and mineralize an ECM, as revealed by alizarin red staining at 7 or 14 days of culture. In contrast, none of the fetal liver-derived stromal cells were found to have the capacity of synthesizing and mineralizing an ECM in basal culture conditions (Fig. 4).

We also investigated the mineralization in response to BMP2 and dexamethasone. Even after 14 days of induction with BMP2 or dexamethasone, No (AFT024) or a very slight (BFC012 and 2012) matrix mineralization was

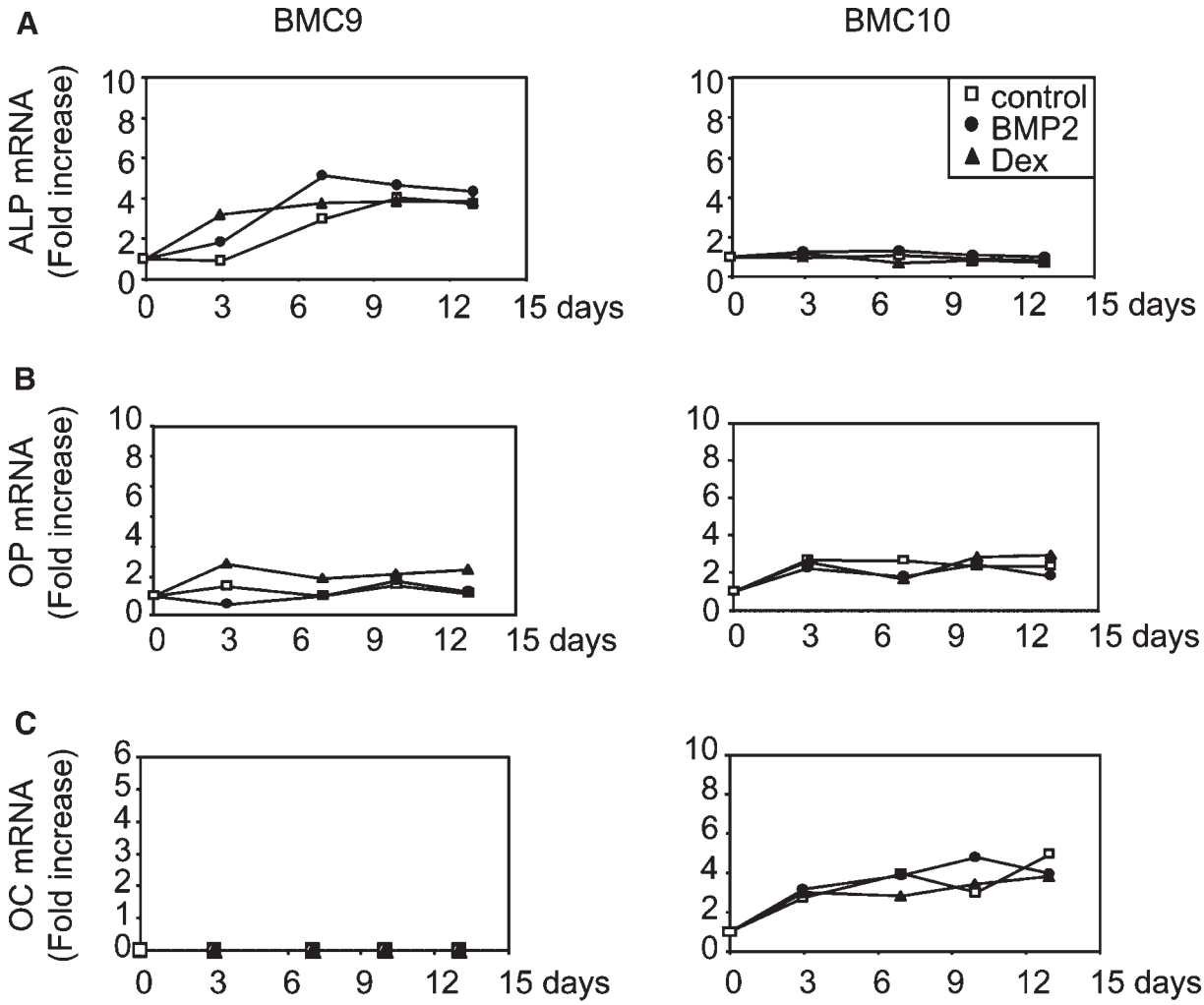

Fig. 2. Expression of osteoblastic markers in bone marrow-derived clonal mesenchymal cells. Bone marrow-derived clonal (BMC9, BMC10) cells were cultured for up to 14 days in the presence or absence of BMP2 (200 ng/ml) or dexamethasone (10^{-7}M), and mRNA levels for early (alkaline phosphatase activity; ALP) (**A**), intermediate (osteopontin; OP) (**B**), and late (osteocalcin; OC) (**C**) osteoblast markers were determined by semi-quantitative RT-PCR. Note that BMC9 and BMC10 cells exhibit a distinct pattern of osteoblast marker gene expression in response to inducers.

observed by day 14 after BMP2 or dexamethasone stimulation (Fig. 4). These results indicate that, under these in vitro conditions, some of fetal liver-derived mesenchymal cells can be induced to express osteoblastic markers under stimulation with osteoblastic differentiation inducers; however, these cells are unable to fully differentiate into mature mineralizing cells.

DISCUSSION

Information on the osteogenic capacity of clonal stromal MSC comes primarily from analysis of murine or human bone marrow stromal MSC [Dennis et al., 1999; Oyajobi et al., 1999; Pittenger et al., 1999]. In contrast, our knowledge is limited on the differentiation capacity of clonal MSC present in non-skeletal hematopoietic compartments. The results of this study indicate that the capacity to mineralize bone marrow-derived lines is superior to that of fetal liver-derived lines, which is in agreement with the microenvironmental difference between the two hematopoiesis sites. However, independent of their site of origin, mesenchymal lines show a marked heterogeneity in their osteoblastic differentiation potential, which further emphasizes the heterogeneity of mesenchymal cells.

One important finding is that some murine clonal fetal liver-derived mesenchymal lines

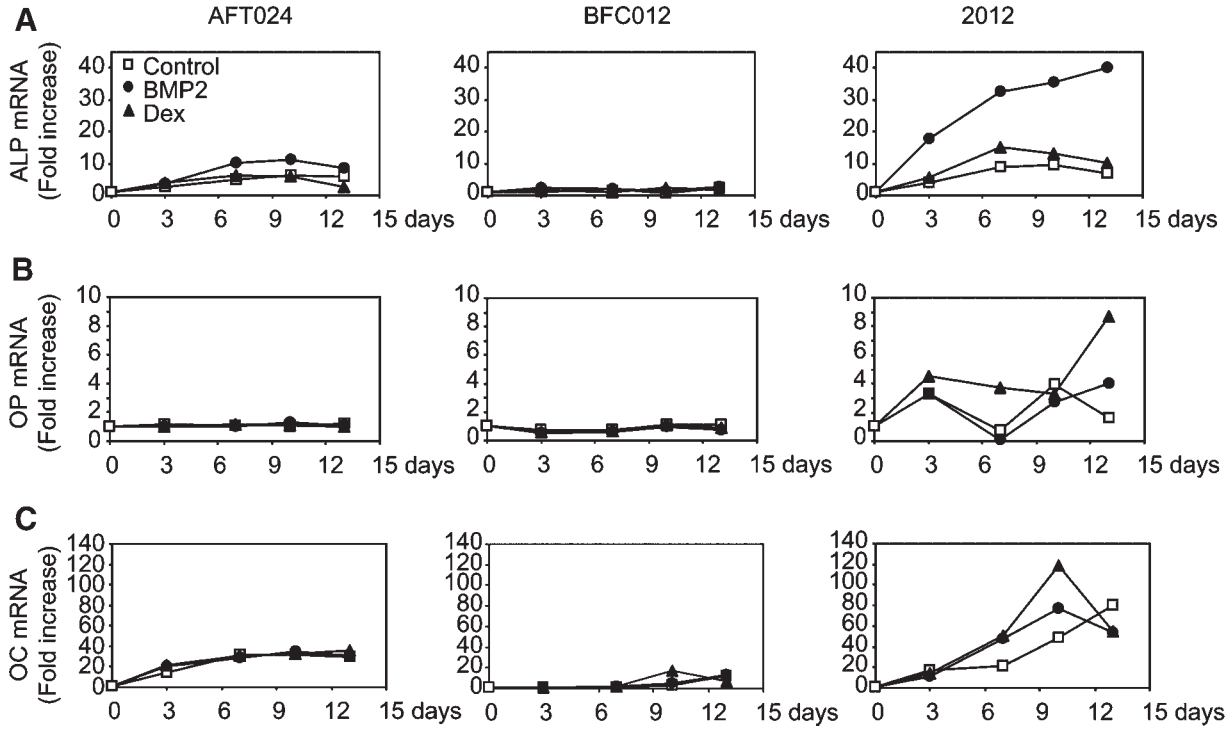

Fig. 3. Effect of osteoblastic differentiation inducers on osteoblastic markers in clonal fetal liver-derived cells. Fetal liver cells (AFT024, BFC012, 2012) were cultured for up to 14 days in the presence or absence of BMP2 (200 ng/ml) or dexamethasone (10^{-7}M), and mRNA levels for the osteoblast markers ALP (**A**), OP (**B**), and OC (**C**) were determined by semi-quantitative RT-PCR. Note that BMP2 or dexamethasone moderately or greatly increased early or late osteoblast markers in AFT024 or 2012 cell lines.

can be induced to express osteoblastic markers such as ALP or osteocalcin under induction by BMP2 or dexamethasone. This indicates that these cells have some ability to differentiate into cells expressing markers of the osteoblastic lineage. ATF024 cells that possess this ability are also able to differentiate into chondrocytes, adipocytes, and vascular smooth muscle cells, contrarily to 2012 or BFC012 which have limited or no differentiation potential [Chateauvieux et al., 2007]. AFT024 cells that are also able to support hematopoiesis [Hackney et al., 2002; Chateauvieux et al., 2007] may derive from a bona fide fetal liver stromal MSC, in contrast to the other lines deriving from mesenchymal precursors with restricted properties. The heterogeneity of the fetal liver-derived lines was also demonstrated by our finding that BMP2 and dexamethasone differently increased the expression of osteoblastic markers such as ALP, OP, and OC in these lines. The different response of clonal lines to these inducers is not specific to MSC from non-skeletal site since bone marrow-derived BMC9 and BMC10 cells also differed in their response to osteogenic stimuli. The different response to the differentiating inducers may result from a distinct basal phenotype such as expression of BMP or glucocorticoid receptors resulting in a variable induction of signaling molecules and transcription factors that are known to mediate the effects of BMP2 and dexamethasone in osteoblast precursor cells [Celil and Campbell, 2005; Osyczka and Leboy, 2005; Phillips et al., 2006; Ito et al., 2007]. Taken together, our results indicate major heterogeneity in the properties of the studied clonal cell lines, which is fitting with the known heterogeneity of MSC according to their proliferation and differentiation potential [Dennis et al., 1999; Oyajobi et al., 1999; Pittenger et al., 1999; Muraglia et al., 2000].

The other major finding reported here is the distinct phenotypic feature that distinguishes fetal liver and bone marrow cells. The complete osteoblast differentiation program is characterized by the production and mineralization of a mineralized ECM [Karsenty and Wagner, 2002]. Bone marrow-derived clonal BMC9 and BMC10 cells were found to produce an

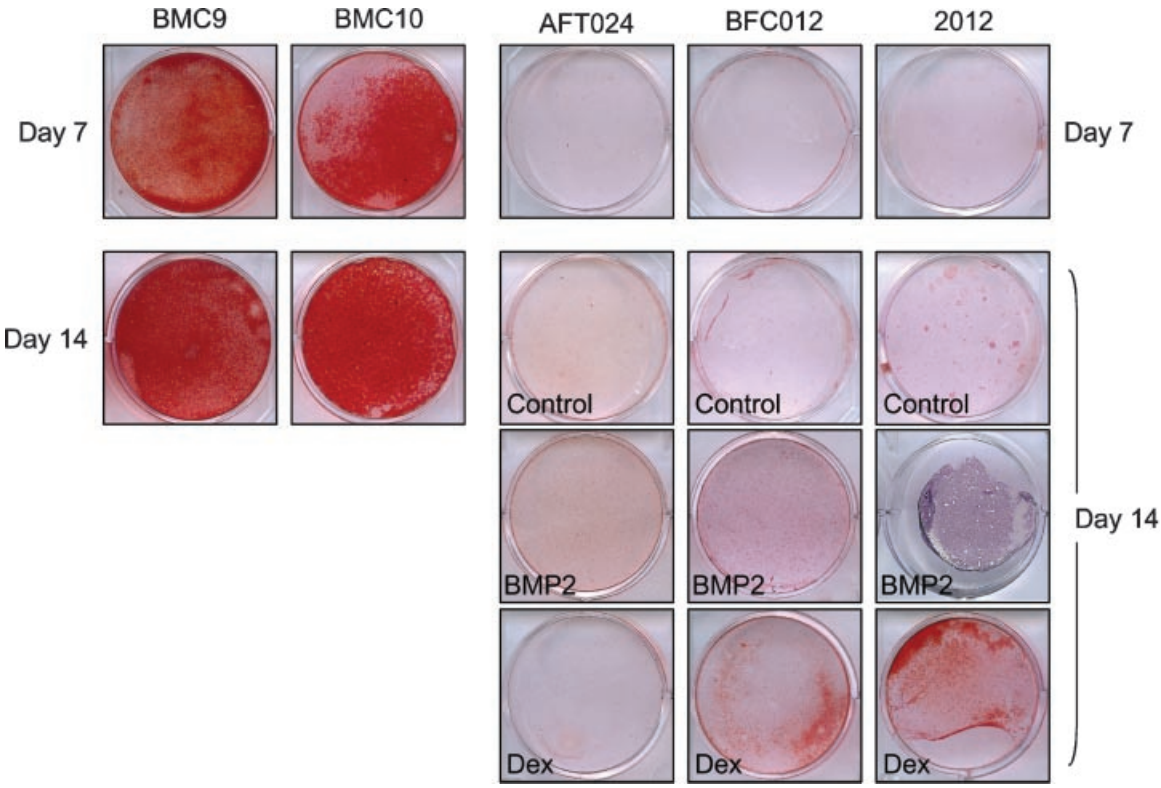

Fig. 4. Distinct mineralization capacity of murine clonal fetal liver and bone marrow mesenchymal cells. Bone marrow-derived clonal (BMC9, BMC10) and fetal liver cells (AFT024, BFC012, 2012) were cultured for up to 14 days in the presence or absence of BMP2 (200 ng/ml) or dexamethasone (10^{-7}M), and the osteogenic capacity was determined by alizarin red staining. Note that, in contrast to bone marrow-derived stromal cells, fetal liver stromal cells show undetectable or little ECM mineralization capacity in vitro.

extensive mineralized matrix, which indicates that the cells are able to behave as functional mature osteoblasts in vitro. In marked contrast, in our conditions, none of the fetal liver-derived stromal cell lines showed significant mineralization in vitro, despite long-term culture in a microenvironment favoring osteogenesis. Early in vivo observations [Marie et al., 1981, 1982] as well as more recent findings [Murshed et al., 2005] indicate that type I collagen, calcium, phosphorus, and ALP activity are required and sufficient to induce bone matrix mineralization in vivo. We found that although ALP activity was induced by BMP2 or dexamethasone in two of the three studied fetal liver-derived cells, the capacity of these cells to produce a mineralized matrix was very limited compared to bone marrow-derived stromal cells, despite the presence of calcium, phosphorus, and ECM formation. This restricted phenotype was not related to the lack of expression of non-collagenous proteins such as osteopontin or osteocalcin, since osteopontin does not contribute but rather inhibits matrix mineralization by osteoblasts [Addison et al., 2007], and osteocalcin is not predictive of the osteogenic potential in vivo [Yamamoto et al., 1991; Ducy et al., 1996]. Overall, these results suggest that intrinsic properties distinguish bone marrow-derived and fetal liver-derived stromal cells in their ability to differentiate into functional mineralizing osteoblasts.

Several hypotheses may be proposed to explain the phenotypic feature that distinguishes MSC from liver and bone marrow stroma. One hypothesis is that the distinct osteogenic capacity of MSC is dependent on the hematopoietic site. In this case, the complete osteogenic differentiation of fetal liver stromal cells may require the action of local factors which are expressed in the bone microenvironment, but not in the fetal liver stroma. This hypothesis is supported by the recent concept that the production of functional mature cells from stem cells is largely dependent on elements of their microenvironment [Moore and Lemischka,

2006]. Additionally, fetal liver cells, which are normally present in a non-skeletal hematopoietic site, may be less sensitive to local osteogenic inducers present in the skeletal environment. Finally, the distinct phenotype observed in fetal liver and bone marrow stromal cells may be due to a distinct developmental program as well as different microenvironment. Recent analysis using cDNA array indicates that gene networks may be operative in MSC independently of the hematopoietic site [Chateauvieux et al., 2007]. The differences in gene expression program in bone marrow and fetal liver cells may explain the preferential differentiation pathway followed by MSC from either site.

REFERENCES

Addison WN, Azari F, Sorensen ES, Kaartinen MT, McKee MD. 2007. Pyrophosphate inhibits mineralization of osteoblast cultures by binding to mineral, up-regulating osteopontin, and inhibiting alkaline phosphatase activity. J Biol Chem 282(21):15872–15883.

Ashton BA, Abdullah F, Cave J, Williamson M, Sykes BC, Couch M, Poser JW. 1985. Characterization of cells with high alkaline phosphatase activity derived from human bone and marrow: Preliminary assessment of their osteogenicity. Bone 6(5):313–319.

Beresford JN, Joyner CJ, Devlin C, Triffitt JT. 1994. The effects of dexamethasone and 1,25-dihydroxyvitamin D3 on osteogenic differentiation of human marrow stromal cells in vitro. Arch Oral Biol 39(11):941–947.

Bianco P, Gehron Robey P. 2000. Marrow stromal stem cells. J Clin Invest 105(12):1663–1668.

Bianco P, Riminucci M, Gronthos S, Robey PG. 2001. Bone marrow stromal stem cells: Nature, biology, and potential applications. Stem Cells 19(3):180–192.

Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. 2001. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 98(8):2396–2402.

Caplan AI. 1991. Mesenchymal stem cells. J Orthop Res 9(5):641–650.

Celil AB, Campbell PG. 2005. BMP-2 and insulin-like growth factor-I mediate Osterix (Osx) expression in human mesenchymal stem cells via the MAPK and protein kinase D signaling pathways. J Biol Chem 280(36):31353–31359.

Chagraoui J, Lepage-Noll A, Anjo A, Uzan G, Charbord P. 2003. Fetal liver stroma consists of cells in epithelial-to-mesenchymal transition. Blood 101(8):2973–2982.

Charbord P, Oostendorp R, Pang W, Herault O, Noel F, Tsuji T, Dzierzak E, Peault B. 2002. Comparative study of stromal cell lines derived from embryonic, fetal, and postnatal mouse blood-forming tissues. Exp Hematol 30(10):1202–1210.

Chateauvieux S, Ichante JL, Delorme B, Frouin V, Pietu G, Langonne A, Gallay N, Sensebe L, Martin MT, Moore KA, Charbord P. 2007. Molecular profile of mouse stromal mesenchymal stem cells. Physiol Genomics 29(2):128–138.

Cheng SL, Yang JW, Rifas L, Zhang SF, Avioli LV. 1994. Differentiation of human bone marrow osteogenic stromal cells in vitro: Induction of the osteoblast phenotype by dexamethasone. Endocrinology 134(1):277–286.

Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. 1999. A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. J Bone Miner Res 14(5):700–709.

Deryugina EI, Muller-Sieburg CE. 1993. Stromal cells in long-term cultures: Keys to the elucidation of hematopoietic development? Crit Rev Immunol 13(2):115–150.

Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, Karsenty G. 1996. Increased bone formation in osteocalcin-deficient mice. Nature 382(6590):448–452.

Dzierzak E. 2001. Haemoglobin disorders. A dynamic system. Lancet 358 (Suppl):S31.

Fromigue O, Marie PJ, Lomri A. 1997. Differential effects of transforming growth factor beta2, dexamethasone and 1,25-dihydroxyvitamin D on human bone marrow stromal cells. Cytokine 9(8):613–623.

Fromigue O, Marie PJ, Lomri A. 1998. Bone morphogenetic protein-2 and transforming growth factor-beta2 interact to modulate human bone marrow stromal cell proliferation and differentiation. J Cell Biochem 68(4):411–426.

Hackney JA, Charbord P, Brunk BP, Stoeckert CJ, Lemischka IR, Moore KA. 2002. A molecular profile of a hematopoietic stem cell niche. Proc Natl Acad Sci USA 99(20):13061–13066.

Hanada K, Dennis JE, Caplan AI. 1997. Stimulatory effects of basic fibroblast growth factor and bone morphogenetic protein-2 on osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells. J Bone Miner Res 12(10):1606–1614.

Ito S, Suzuki N, Kato S, Takahashi T, Takagi M. 2007. Glucocorticoids induce the differentiation of a mesenchymal progenitor cell line, ROB-C26 into adipocytes and osteoblasts, but fail to induce terminal osteoblast differentiation. Bone 40(1):84–92.

Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. 1997. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 64(2):295–312.

Kamalia N, McCulloch CA, Tenebaum HC, Limeback H. 1992. Dexamethasone recruitment of self-renewing osteoprogenitor cells in chick bone marrow stromal cell cultures. Blood 79(2):320–326.

Karsenty G, Wagner EF. 2002. Reaching a genetic and molecular understanding of skeletal development. Dev Cell 2(4):389–406.

Kassem M. 2004. Mesenchymal stem cells: Biological characteristics and potential clinical applications. Cloning Stem Cells 6(4):369–374.

Lee KS, Kim HJ, Li QL, Chi XZ, Ueta C, Komori T, Wozney JM, Kim EG, Choi JY, Ryoo HM, Bae SC. 2000. Runx2 is a common target of transforming growth factor beta1 and bone morphogenetic protein 2, and cooperation between Runx2 and Smad5 induces osteoblast-specific gene expression in the pluripotent mesenchymal precursor cell line C2 C12. Mol Cell Biol 20(23):8783–8792.

Luppen CA, Smith E, Spevak L, Boskey AL, Frenkel B. 2003. Bone morphogenetic protein-2 restores mineralization in glucocorticoid-inhibited MC3T3-E1 osteoblast cultures. J Bone Miner Res 18(7):1186–1197.

Malaval L, Modrowski D, Gupta AK, Aubin JE. 1994. Cellular expression of bone-related proteins during in vitro osteogenesis in rat bone marrow stromal cell cultures. J Cell Physiol 158(3):555–572.

Marie PJ, Fromigue O. 2006. Osteogenic differentiation of human marrow-derived mesenchymal stem cells. Regen Med 1(4):539–548.

Marie PJ, Pettifor JM, Ross FP, Glorieux FH. 1982. Histological osteomalacia due to dietary calcium deficiency in children. N Engl J Med 307(10):584–588.

Marie PJ, Travers R, Glorieux FH. 1981. Healing of rickets with phosphate supplementation in the hypophosphatemic male mouse. J Clin Invest 67(3):911–914.

Marom R, Shur I, Solomon R, Benayahu D. 2005. Characterization of adhesion and differentiation markers of osteogenic marrow stromal cells. J Cell Physiol 202(1): 41–48.

Moore KA, Lemischka IR. 2006. Stem cells and their niches. Science 311(5769):1880–1885.

Muguruma Y, Yahata T, Miyatake H, Sato T, Uno T, Itoh J, Kato S, Ito M, Hotta T, Ando K. 2006. Reconstitution of the functional human hematopoietic microenvironment derived from human mesenchymal stem cells in the murine bone marrow compartment. Blood 107(5):1878–1887.

Muraglia A, Cancedda R, Quarto R. 2000. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 113(Pt 7):1161–1166.

Murshed M, Harmey D, Millan JL, McKee MD, Karsenty G. 2005. Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ECM mineralization to bone. Genes Dev 19(9):1093–1104.

Oreffo RO, Cooper C, Mason C, Clements M. 2005. Mesenchymal stem cells: Lineage, plasticity, and skeletal therapeutic potential. Stem Cell Rev 1(2):169–178.

Osyczka AM, Leboy PS. 2005. Bone morphogenetic protein regulation of early osteoblast genes in human marrow stromal cells is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase signaling. Endocrinology 146(8):3428–3437.

Owen M. 1988. Marrow stromal stem cells. J Cell Sci Suppl 10:63–76.

Oyajobi BO, Lomri A, Hott M, Marie PJ. 1999. Isolation and characterization of human clonogenic osteoblast progenitors immunoselected from fetal bone marrow stroma using STRO-1 monoclonal antibody. J Bone Miner Res 14(3):351–361.

Phillips JE, Gersbach CA, Wojtowicz AM, Garcia AJ. 2006. Glucocorticoid-induced osteogenesis is negatively regulated by Runx2/Cbfa1 serine phosphorylation. J Cell Sci 119(Pt 3):581–591.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. 1999. Multilineage potential of adult human mesenchymal stem cells. Science 284(5411): 143–147.

Prockop DJ. 1997. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276(5309):71–74.

Qi H, Aguiar DJ, Williams SM, La Pean A, Pan W, Verfaillie CM. 2003. Identification of genes responsible for osteoblast differentiation from human mesodermal progenitor cells. Proc Natl Acad Sci USA 100(6):3305–3310.

Quarles LD, Yohay DA, Lever LW, Caton R, Wenstrup RJ. 1992. Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: An in vitro model of osteoblast development. J Bone Miner Res 7(6):683–692.

Rickard DJ, Sullivan TA, Shenker BJ, Leboy PS, Kazhdan I. 1994. Induction of rapid osteoblast differentiation in rat bone marrow stromal cell cultures by dexamethasone and BMP-2. Dev Biol 161(1):218–228.

Rickard DJ, Kassem M, Hefferan TE, Sarkar G, Spelsberg TC, Riggs BL. 1996. Isolation and characterization of osteoblast precursor cells from human bone marrow. J Bone Miner Res 11(3):312–324.

Shui C, Spelsberg TC, Riggs BL, Khosla S. 2003. Changes in Runx2/Cbfa1 expression and activity during osteoblastic differentiation of human bone marrow stromal cells. J Bone Miner Res 18(2):213–221.

Thiemann FT, Moore KA, Smogorzewska EM, Lemischka IR, Crooks GM. 1998. The murine stromal cell line AFT024 acts specifically on human CD34 + CD38- progenitors to maintain primitive function and immunophenotype in vitro. Exp Hematol 26(7):612–619.

Yamamoto T, Ecarot B, Glorieux FH. 1991. In vivo osteogenic activity of isolated human bone cells. J Bone Miner Res 6(1):45–51.

Yin T, Li L. 2006. The stem cell niches in bone. J Clin Invest 116(5):1195–1201.

Zipori D. 1989. Cultured stromal cell lines from hematopoietic tissues. In: Tavassoli M, editor. Handbook of the hemopoietic microenvironment. Clifton, New Jersey: Humana Press. pp. 287–333.