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Identification of CD13⁺CD36⁺ cells as a common progenitor for erythroid and myeloid lineages in human bone marrow

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Abstract

Objective—To identify bi-potential precursor cells of erythroid and myeloid development in human bone marrow.

Materials and Methods—Cells co-expressing CD13 and CD36 (CD13⁺CD36⁺) were investigated by analyzing cell surface marker expression during erythroid development (induced with a combination of cytokines plus erythropoietin [EPO]), or myeloid development (induced with the same cocktail of cytokines plus granulocyte-colony stimulating factor [G-CSF]) of bone marrow derived CD133 cells in liquid cultures. CD13⁺CD36⁺ subsets were also isolated on the 14th day of cultures and further evaluated for their hematopoietic clonogenic capacity in methylcellulose.

Results—Colony-forming analysis of sorted CD13⁺CD36⁺ cells of committed erythroid and myeloid lineages demonstrated that these cells were able to generate erythroid, granulocyte, and mixed erythroid–granulocyte colonies. In contrast, CD13⁺CD36[−] or CD13[−]CD36⁺ cells exclusively committed to granulocyte/monocyte or erythroid colonies, respectively, but failed to form mixed erythroid–granulocyte colonies; no colonies were detected in CD13[−]CD36[−] cells with lineage-supporting cytokines. In addition, our data confirmed that EPO induced both erythroid and myeloid commitment, while G-CSF only supported the differentiation of the myeloid lineage.

Conclusions—The present data identify some CD13⁺CD36⁺ cells as bi-potential precursors of erythroid and myeloid commitment in normal hematopoiesis. They provide a physiological explanation for the cell identification of myeloid and erythroid lineages observed in hematopoietic diseases. This unique fraction of CD13⁺CD36⁺ cells may be useful for further studies on regulating erythroid and myeloid differentiation during normal and malignant hematopoiesis.

INTRODUCTION

All types of blood cells, including erythroid and myeloid cells, begin at the level of pluripotent hematopoietic stem/progenitor cells [1]. The hematopoietic stem/progenitor cells have been well-characterized in the past, but the understanding of defined committed lineage cells is relatively limited. Multi-lineage development from single hematopoietic stem/progenitor cell raises the possibility that multi- or bi-potential lineage cells may exist at various branches of

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lineage development and that these cells may be able to respond under local conditions to determine their fate. Previous studies have suggested that erythroid and myeloid lineages share common precursor cells (common myeloid progenitors) giving rise to all types of myeloid cells and erythroid cells without lymphocytes [2,3]. A recent report has shown that common myeloid progenitors can be isolated from normal bone marrow using antibodies to cell surface markers, including CD19, CD34, CD45RA, IL-3R α , and thrombopoietin receptor [4]. On the other hand, in some types of hematopoietic disorders, such as transient myeloproliferative disorder (TMD), myelodysplastic syndromes (MDS), and some leukemias, abnormalities of the erythroid and myeloid development often accompany each other. All of these diseases appear to exhibit a mixed expression of myeloid- and erythroid-specific markers. The promiscuous expression of these lineage-specific markers has so frequently been observed in hematopoietic malignancies [5,6] that it is considered a hallmark of leukemia [5]. Furthermore, the switch between erythroid and myeloid lineages has also been reported on some transformed hematopoietic [7–9] and leukemic [10–12] cell lines. Taken together, these studies suggest that erythroid and myeloid development are closely associated in both normal hematopoiesis and hematopoietic disorders.

We have previously shown that when CD133 (also known as AC133)-selected hematopoietic stem/progenitor cells are induced to follow the erythroid developmental pathway using erythropoietin (EPO) treatment, the resulting cells can still be redirected to develop into myeloid cells when EPO is replaced by granulocyte-colony stimulating factor (G-CSF), or vice versa [13–15]. These results indicate that some bi-potential precursors may exist in EPO-induced erythroid or G-CSF-induced myeloid populations [16,17]. Isolation of hematopoietic cells at committed stages and study of their physiological processes will add to our understanding of the developmental features of hematopoietic stem/progenitor cells and the origin of hematopoietic malignancies [18]. In the present study, we tested the hypothesis that bi-potential precursors exist in committed erythroid and myeloid lineage cells induced from hematopoietic stem/progenitor cells. Because CD13 and CD36 are commonly used as myeloid- or erythroid-specific markers, respectively, we observed the expression of these two markers and other antigens during 28-day cultures of hematopoietic stem/progenitor cells induced by EPO or G-CSF. Changes in lineage marker expression for cells cultured under these two conditions were monitored. We also sorted CD13⁺CD36⁺, CD13⁺CD36⁻, CD13⁻CD36⁺ cells and investigated their capacity to further differentiate when exposed to different induction agents. The results demonstrate the existence of the bi-potential ability of CD13⁺CD36⁺ cells for developing into erythroid and myeloid development in defined culture conditions. In addition, our data confirm that EPO induced both erythroid and myeloid differentiation, whereas G-CSF only supported myeloid commitment.

MATERIALS AND METHODS

Liquid culture system for cell differentiation

Human bone marrow CD133⁺ cells (AllCells, Berkeley, CA, USA) were cultured for 14–28 days in the presence of EPO or G-CSF. All cells were grown in media that included stem cell factor (SCF, 50 ng/mL), interleukin-3 (IL-3, 10 ng/mL), granulocyte-macrophage colony-stimulating factor (GM-CSF; 10 ng/mL), and 30% fetal bovine serum (HyClone, MN, USA) as described previously [14]. All growth factors are purchased from Stem Cell Technologies (Vancouver, BC, Canada). Alternatively, cells were exposed to either EPO or G-CSF for various time-courses up to 4 weeks. D0 (Day zero) is defined as the starting date. Control cells were grown in the same medium free of cytokines.

Analysis of cell phenotype

A wide panel of cell differentiation markers was used to analyze the cultured cells at timepoints during culture (Day0, Day7, Day14, Day28). Each group at each timepoint was analyzed by

fluorescence-activated cell sorting (FACS) with a FACS Calibur flow cytometer and CellQuest software (Becton Dickinson, San Jose, CA, USA). Phycoerythrin [PE]-conjugated anti-CD133 antibody was obtained from Miltenyi Biotec (Auburn, CA, USA). All other antibodies against cell surface markers (CD34, CD71, CD36, glycophorin A, CD13, CD33, CD2, and CD19) were from BD Biosciences-Pharmingen (San Diego, CA, USA).

CD133⁺CD36⁺ cell selection

CD133⁺ cells cultured for 14 days with EPO or G-CSF still retained the expression of both CD36⁺ and CD13⁺. To identify population(s) that contain common precursors for both myeloid and erythroid development, these cells were sorted based on their expression of CD13 and CD36 using the FACS Vantage system (Becton Dickinson). Sorted cells were assessed morphologically by Wright-Giemsa staining as described previously [14].

Colony-forming assay

The sorted hematopoietic cells were plated in 35-mm culture dishes in methylcellulose media (MethoCult GF H4434, H4535, H4435, or MethoCult SFBIT H4236; StemCell Technologies, Vancouver, BC, Canada). The MethoCult GF H4434 medium (for erythroid lineage development) consists of EPO, stem cell factor, GM-CSF, and IL-3; H4535 (for myeloid lineage) of G-CSF, Stem Cell Factor, GM-CSF, IL-3, and IL-6; and H4435 (for both erythroid and myeloid lineages) of EPO, G-CSF, GM-CSF, IL-3, and IL-6. The medium H4236 as a control includes serum-free methylcellulose medium without any cytokines. Interleukin-6 (IL-6; 20 ng/mL) was added into H4434 media to match the composition of H4535 and H4435 media. Colony-forming cultures were performed in duplicate in three independent experiments. At Day 12–14, colonies containing more than 50 cells were counted and classified as granulocyte colony, monocyte/macrophage colony, erythroid blast colony, or mixture of granulocytes and erythroid colony, and colonies containing 20–50 cells of erythroid colony with a similar method as described previously [13]. The colonies were counted by standard criteria in 60-mm gridded scoring dishes with cross marks (Stem Cell Technologies) under an inverted microscope. At this time, macroscopic red erythroid colonies were readily distinguishable from purely granulopoietic colonies. Macrophage colonies could be recognized by the presence of large macrophage cells. A colony with a single cell center was counted as an individual colony. In some case, although it happened rarely, a large colony was connected to or even covered other colony or colonies forming multiple cell centers, such joined colonies were scored as several individual colonies to make sure there is minimal possibility for false mixed colonies. Single colonies were picked from the semisolid medium with a fine pipette and collected in Eppendorf microcentrifuge tubes containing 50–100 μ l (dependent on the size of colonies) PBS. The individual colony suspensions were cytocentrifuged (Cytospin, Shandon, Cheshire, U.K.) at 2000 rpm for 2 min onto SILANATENTTM slides (Digene, Beltsville, MD), and stained with Wright–Giemsa staining. The cellular composition of each colony was determined morphologically under the microscope.

RESULTS

Morphologies of CD133⁺ hematopoietic stem/progenitor cells under different culture conditions

The morphologic changes that occurred during the culture of CD133⁺ hematopoietic stem/progenitor cells in the presence of EPO or G-CSF are shown in Figure 1. CD133⁺ cells displayed morphological characteristics of undifferentiated blast cells (such as small cell size and high nuclear density) at D0. Cells cultured for 14 days with EPO (E14) or G-CSF (G14) appeared to be largely erythroid or myeloid cells, respectively. Cultures exposed to EPO for 28 days (E28) contained an average 74% erythroid cells and 26% granulocytes/monocytes. The same starting CD133⁺ hematopoietic stem/progenitor cells cultured with G-CSF for 21–

28 days (G21, G28) possessed almost 100% granulocytes/monocytes, without detectable erythroid cells. Cells cultured without EPO or G-CSF and other cytokines proliferated slowly and most were observed to go on to become granulocytes or monocytes after 28 days of culture, with no erythroid cells observed.

FACS analysis of CD13⁺CD36⁺ population

Changes in cell surface marker expression were observed during erythroid and myeloid development of CD133⁺ cells in the presence of cytokines (Fig. 2). At D0, a 26% of cells co-expressed CD13 and CD36 markers (Fig. 2Aa). Under erythroid commitment culture conditions (EPO treatment), the percentage of CD13⁺CD36⁺ cells increased to 72% at E14 (Fig. 2Ac), and then decreased to 37% at E28 (Fig. 2Ae). By contrast, under culture conditions favouring myeloid (granulocytes and monocytes) differentiation (G-CSF treatment), cells co-expressing CD13 and CD36 decreased from 26% at D0 (Fig. 2Aa) to 13% at G14 (Fig. 2Bc), and then increased to 37% at G28 (Fig. 2Be). Interestingly, the majority of CD71⁺ (transferrin receptor) cells co-expressed CD36 markers after exposure to EPO (E14; Fig. 2Ad), while the CD71⁺ cells less co-expressed CD36 (G14; Fig. 2Bd), but largely co-expressed CD13 antigen with G-CSF treatment (data not shown).

CD133 expression on the initial population of bone marrow cells (>97%, Fig. 2Ba, Bb) decreased over time as they differentiated into cells more committed to a particular lineage. Fewer than 1% of E14 or G14 cells were CD133⁺ (data not shown) in 14-day cultures. The CD34 marker persisted slightly longer than CD133, but also dropped to less than 1% in E14 or G14 population cells (data not shown). The expression of CD33 antigen was consistent with expression of CD13 marker in almost all populations of cells observed (data not shown). A limited number of CD2⁺ or CD19⁺ cells were encountered on D0 (Fig. 2Ba, Bb); they became undetectable at about the 7th day of culture with EPO or G-CSF (data not shown).

The distribution of the different fractions of G14 and E14 cells is indicated in Fig 3A. The CD13⁺CD36⁺ subset cells showed 4% in G14 and 51% in E14 population cells, respectively. CD13⁺ cells were the majority of cells in G14 and 4 times more than the cells in E14, while the percentage of CD36⁺ cells were much higher (> 50 times) in E14 than in G14 cells, reflecting cytokine (G-CSF or EPO) specific and lineage differentiation in the culture system.

Isolation of CD13⁺CD36⁺ subset

After 14 days of culture under conditions inducing committed erythroid (E14) or myeloid (G14) lineages, cells were sorted based on their expression of CD13 and/or CD36 (Fig. 3). The morphology of sorted cells is shown in Fig. 3B. CD13⁺ cells sorted from both G14 and E14 cultures showed morphologic characteristics of the myeloid phenotype, while CD36⁺ cells from either G14 or E14 cultures exhibited properties of erythroid cells. However, cells co-expressing CD13 and CD36 markers from G14 cultures revealed a different morphology from the CD13⁺CD36⁺ cells from E14 culture. The former subset appeared to more closely resemble myeloid morphology, whereas the latter appeared to be early erythroid progenitors. Too few CD13⁺CD36⁺ cells (data not shown) were obtained by sorting to draw any conclusion about this subpopulation in these studies.

In vitro differentiation potential of CD13⁺CD36⁺ cells

To investigate the potential of CD13⁺CD36⁺ cells and other cultured cells to differentiate into myeloid and erythroid lineages, we examined their colony-forming ability. Table 1 summarizes the colony numbers and types formed by these sorted cells when analyzed in colony-forming assays. CD13⁺CD36⁺ subsets isolated from E14 and G14 cultures generated erythroid, myeloid, and mixed erythroid –granulocyte colonies in the presence of EPO or EPO + G-CSF. It is interesting that the mixed erythroid –granulocyte colonies were only induced from

CD13⁺CD36⁺ cells with EPO + G-CSF or EPO alone, indicating these cells possess bi-potential for erythroid and myeloid development and EPO could stimulate both erythroid and myeloid development. Erythroid outgrowth was not observed in CD13⁺CD36⁺ cells in the presence of G-CSF only, suggesting that G-CSF could not support CFU-E or BFU-E growth.

CD13⁺CD36⁺ cells from both G14 and E14 cultures exclusively differentiated into erythroid colonies in the presence of EPO or EPO + G-CSF. Only a few granulocyte colonies appeared from E14 cells cultured with EPO + G-CSF, indicating that the CD13⁺CD36⁺ population loses myeloid (granulocyte and monocyte) development potential. In our studies, CD13⁺CD36⁺ cells from G14 and E14 cultures mostly differentiated into myeloid colonies, while a few of them committed into erythroid cells in the presence of EPO or EPO + G-CSF, presumably reflecting that some cells of this population may have the potential for erythroid commitment with EPO stimulation. No colonies were detected in CD13⁺CD36⁺ cells at various culture conditions in the cell number range we examined.

DISCUSSION

In this study, we have isolated the CD13⁺CD36⁺ subset of cells from human bone marrow CD133⁺ stem/progenitor cells for both myeloid and erythroid lineage commitment. CD13 is a unique myeloid antigen expressed as early as the blast stage of lineage development and at high levels on myeloblasts and promyelocytes [19,20]. CD36 has been used as an early erythroid marker [21,22], although it is also expressed on late megakaryocytic and monocytic cells [23]. Indeed, a high proportion of CD36⁺ progenitors only generated erythroid burst-forming unit (BFU-E) and erythroid colony-forming units (CFU-E) [23,24]. In a recent paper, Singh *et al.* [25] also showed that purified subpopulations of CD34⁺CD36⁺ and CD34⁺CD36⁺ cells from baboon bone marrow generated mostly BFU-E and CFU-E colonies. To date, few published studies have directly examined CD13⁺CD36⁺ subset cells in a steady state or under culture conditions. Our data showed a 23% of CD133⁺ cells at D0 (without EPO or G-CSF cytokine stimulation) were CD13⁺CD36⁺ [Fig.2, Aa]. This value agrees with the studies by Oertel *et al.* [26] and by Thoma *et al.* [27], in which, the expression of CD13 was 33±15% and 70±13%, and the expression of CD36 was 22±8% and 6±3% on human bone marrow and cord blood, respectively. Our present study and above work [26] showed that cells expressing CD13 and CD36 committed to myeloid and erythroid colonies, respectively, but no lymphocytes were detected in these colonies. In our study, in the presence of cytokines, CD133⁺ cells co-expressing CD13 and CD36 increased from 26% at D0 to 72% after culture for 14 days in EPO (E14), but decreased to 13% after culture for 14 days in G-CSF (G14). Meanwhile, in both E14 and G14 cultures, CD133⁺CD13⁺CD36⁺ or CD34⁺CD13⁺CD36⁺ cells decreased to less than 1% from their original >97% at D0, which is consistent with a previous report [28]. It is unlikely that the minimal number of CD133⁺CD13⁺CD36⁺ or CD34⁺CD13⁺CD36⁺ cells at D14 can give rise to abundant erythroid or myeloid lineage cells at this point [14]. However, some of the CD13⁺CD36⁺ cells may still express a low intensity of known stem cell markers (such as CD133 and CD34) or other unexpected marker(s). The CD13⁺CD36⁺ cells may represent an early branch of precursor cells, and have at least bi-differentiation potential for erythroid and myeloid development. It is notable that the increment of the CD13⁺CD36⁺ subset observed in G28 cells (up to 37% from 13% at G14) may largely reflect the expression of CD36 on late monocytes in this population of cells.

The observation of CD13⁺CD36⁺ cells in E14 (72%) and G14 (13%) confirms that cells co-expressing both myeloid and erythroid lineage-specific markers do exist. The colony-forming experiments further demonstrate this bi-directional differentiation potential. Co-expression of CD13 and CD36 antigens might reflect the intracellular overlap or balance of transcription factors, such as C/EBPα [7], GATA-1 and PU.1 [29], or other lineage switch-related genes, such as Myb LZR [30] and glia maturation factor gamma [15] although the molecular

mechanisms controlling erythroid and myeloid commitment are largely unknown. These transcription factors or genes may function as a base on these cells of possessing bi-potential differentiation for their fate conversion (switching) or lineage plasticity in normal hematopoiesis.

In our studies, granulocyte commitment was always accompanied by monocyte colonies when $CD13^+CD36^+$ or $CD13^+CD36^-$ cells were induced for erythroid or myeloid cell differentiation, whereas myeloid lineage was not detected in $CD13^-CD36^+$ cells stimulated with EPO and/or G-CSF. These results indicate a sequence of hematopoietic lineage commitment, in which progenitors first generate myeloid lineage cells, followed by monocytes, and then erythroid cells under certain conditions [Fig. 4]. The results are consistent with a previous report on the order of myeloid and erythroid development from HSCs [31].

In some hematopoietic disorders, such as the transient myeloproliferative disorder (TMD), acute myeloid leukemia (AML), and myelodysplastic syndromes (MDS), abnormal cells occur in more than one lineage. TMD is an abnormal proliferation of myeloid blasts in the blood that rapidly undergoes spontaneous remission without therapeutic intervention [32]. A high percentage of TMD patients will develop AML [33], so it is also considered as a pre-leukemic condition. CD36 antigen has been reported being expressed in all TMD patients [34]. A subpopulation of immature blast cells expressing cross-lineage antigens, including CD13 and CD36, are observed in TMD, indicating the erythroid development of TMD even in the absence of $CD34^+$ cells [35]. Erythroid differentiation in blast cells of TMD is further demonstrated by erythroid-specific gene expression [36]. AML is a heterogeneous group of leukemias in which myeloid maturation is blocked at different differentiation stages, resulting in single- or mixed-cell lineage leukemic blasts. Thus, almost all AML cells express CD13, CD33, and CD117 [37], of which most co-express CD36 [34]. Furthermore, the expression of CD36 antigen on AML cells is related to a higher risk of relapse and a lower leukemia-free survival, and has been suggested as a key marker for an adverse prognosis irrespective of genetic abnormalities [37]. MDS are also heterogeneous diseases of bone marrow cell precursors, with five categories including RAEB (refractory anemia with excess of blasts) [38]. High expression of CD36, which was not associated with the expression of CD34 or CD71 in RAEB [39], is an indicator of poor prognosis in patients with MDS.

These observations indicate that precursors expressing CD36 and CD13 could be involved in spontaneous recoveries or/and leukemic transformation processes in hematopoietic disorders. Such a mechanism would suggest that cells co-expressing CD36 and CD13 may represent a unique subgroup, which is independent of CD34 or CD71 in blast cells of these diseases [35, 39]. CD71 has been described as an erythroid antigen. The relationship between CD71 expressed levels on CD34 cells and erythroid and myeloid development has been reported by Mayani et al, showing that CD71 antigen was expressed at high level on CD34+ cells committing to the erythroid development, but at low level on CD34+ cells differentiating into the myeloid lineage [40]. It is possible that CD34 or/and CD71 are expressed at a low levels on some $CD13^+CD36^+$ cells of bi-potential precursors. Thus, some conditions affecting exclusively one cell type with some abnormalities in the other cell lineage, such as Shwachman-Diamond neutropenia, may possibly spontaneously recover by employing these unique bi-directional cells. In malignant hematopoiesis, on the other hand, leukemic cells may be blocked at such stages and thus lose their ability for further differentiation. The special phenotype of cancer cells may in part be derived from the intrinsic properties of such bi-potential hematopoietic precursor cells. This hypothesis regarding the role of bi-potential precursors is supported by a recent report of a new erythroleukemic cell line, ERY-1, which possesses both the myeloid and erythroid markers CD13, CD33, CD36, and glycophorin A [6].

EPO and G-CSF are major growth factors for inducing the proliferation and differentiation of erythroid and myeloid progenitor cells, respectively. Our data showed that the proliferation and differentiation of erythroid and myeloid phenotypes could be induced from the same source of CD133⁺ cells, which is consistent with the effects of these cytokines on hematopoiesis [41,42]. Our observation of semi-solid cultures allows us to directly monitor the effect of EPO and G-CSF on the myeloid and erythroid lineage branching-point. The analysis of sorted CD13⁺CD36⁺, CD13⁺CD36⁻, or CD13⁻CD36⁺ cells from EPO- and G-CSF-treated CD133⁺ cells (E14, G14) confirms that EPO supports both erythroid [41,43] and myeloid [44] differentiation. This conclusion is drawn from the special setting in our experiments, in which fetal bovine serum and other cytokines (SCF, IL-3, IL-6, and GM-CSF) were included. This bi-effect of EPO on erythroid and myeloid differentiation provides for the possibility that inter-conversion or switching of erythroid and myeloid lineages during their development could occur. In contrast, G-CSF had no significant effect on erythroid progenitors in G14, consistent with previous data [45]. The differential effects of EPO and G-CSF on erythroid and myeloid development may in part explain the observations in switch-culture experiments done previously [13–15], in which E14 cells contain a number of CD13⁺ cells, rather than being exclusively committed to erythroid lineage, while G14 cells treated with EPO for another 14 days instead of G-CSF still retained some myeloid progenitor cells.

In conclusion, this is the first report on a subset of cells co-expressing CD13 and CD36 from human bone marrow. The CD13⁺CD36⁺ cells retain at least ‘bi-potential’ capacity of committing to erythroid and myeloid lineages in the presence of EPO or G-CSF. Thus, the identification of this specific subpopulation confirms the hypothesis of the existence of bi-potential cells in a relatively late committed lineage and provides a physiological basis for the phenomena of cross-lineage marker expression observed in hematopoietic malignancies [5]. It also indicates that co-expression of erythroid- and myeloid-specific markers (e.g., CD36 and CD13) may be an intrinsic feature of normal hematopoiesis, rather than simply a hallmark of hematopoietic malignancies. More importantly, the subpopulation of CD13⁺CD36⁺ precursor cells, which are not transformed, may be used for further studies of the regulatory mechanisms controlling erythroid and myeloid development pathways underlying normal and leukemic hematopoiesis.

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Figure 1. *In vitro* differentiation and maturation of erythroid and myeloid lineages derived from CD133 cells

Human bone marrow stem/progenitor cells (D0, 97% purity) were cultured in the presence of a cytokine mixture containing erythropoietin (EPO) or granulocyte-colony stimulating factor (G-CSF) for 28 days. Cells harvested from day 14 to day 28 showed the erythroid- and myeloid-enriched population cells induced by EPO or G-CSF, respectively. The numbers under each micrograph show the distribution of erythroid (in red), granulocyte (in green), and monocyte (in black) cells in a particular population. For each sample of each experiment, 500 to 600 cells were evaluated microscopically with Giemsa staining (original magnification, x200). The scale bar in D0 image of CD133 cells indicates a length of 10 μ m. SCF, stem cell factor; IL-3, interleukin-3; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Figure 2. Flow cytometry analysis of cells co-expressing CD13 and CD36 antigens as markers of erythroid and myeloid development of CD133⁺ cells

Surface antigen CD36 (indicative of erythroid differentiation) and CD13 (indicative of myeloid differentiation) in human bone marrow CD133⁺ hematopoietic stem/progenitor cells cultured at 14 and 28 days with EPO (E14, E28) or with G-CSF (G14, G28) were analyzed by flow cytometry. Cells were stained with combinations of CD13 (PE), CD36 (FITC), CD71 (PE-Cy5), CD133 (PE), CD19 (FITC), and CD2 (PE-Cy5) as indicated. CD13 and CD36 markers are differentially co-expressed on cell populations as erythroid and myeloid populations differentiated and matured. CD2 and CD19 markers appeared only at D0 in a limited percentage of cells (Ba, Bb).

Figure 3. Cell sorting of the CD13⁺CD36⁺ subset of 14-day cultures with G-CSF or EPO stimulation
 A. Cell sorting of CD13⁺CD36⁺ subset. After culturing for 14 days with EPO (E14) or G-CSF (G14) treatment, cells expressing CD13⁺CD36⁻, CD13⁻CD36⁺, CD13⁺CD36⁺, and CD13⁻CD36⁻ were sorted. B. Morphology of sorted cell populations. CD13⁺CD36⁺ cells appeared to be a majority population in E14, and a small fraction in G14. The CD13⁺CD36⁺ cells derived from G14 appeared morphologically to be committed to myeloid lineage, whereas the CD13⁺CD36⁺ cells from E14 appeared morphologically to be committed to the pathway to erythroid development. On the other hand, CD13⁺CD36⁻ and CD13⁻CD36⁺ cells from both G14 and E14 possessed a similar morphology.

Figure 4.

Schematic representation of lineage-specific antigen expression of erythroid and myeloid development and cytokine stimulation *in vitro*. Lineage-specific markers of erythroid (CD36, in red), myeloid (CD13, in green), and progenitor (CD133, in yellow) cells are expressed on cells of single- (CD13⁺ or CD36⁺ subset, in green or red square) or bi-potential (CD13⁺CD36⁺ subset, in yellow circle) treated with EPO (arrow in red) and G-CSF (arrow in green) and sorted from 14 day's liquid cultures, respectively. The maturation of myeloid and erythroid lineages is characterized by the appearance of CD13 and CD36 from CD133⁺ hematopoietic stem/progenitor cells. Isolated CD13⁺CD36⁺ precursor cells possess the potential to be further differentiated into myeloid or/and erythroid cells in presence of EPO, G-CSF, and EPO+G-CSF in methylcellulose.

Table 1
Growth of colonies generated from sorted cells of G-CSF-(G14) and EPO-stimulated population (E14)

Cells	Cytokines in methylcellulose					CD13 ⁺ CD36 ⁻ * (4.0×10 ⁴ /mL)				CD13 ⁺ CD36 ⁺ * (4.0×10 ⁴ /mL)				CD13 ⁻ CD36 ⁻ * (4.0×10 ⁴ /mL)			
	GM-CSF	IL-3	IL-6	EPO	G-CSF	E	G	E/G	M	E	G	E/G	M	E	G	E/G	M
G14	+	+	+	+	-	3	118	0	20	220	0	0	0	19	2	3	1
	+	+	+	-	+	0	160	0	47	0	0	0	0	0	8	0	14
	+	+	+	+	+	1	194	0	61	200	0	0	0	16	7	7	10
	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
E14	+	+	+	+	-	2	80	0	64	552	0	0	0	168	11	5	2
	+	+	+	-	+	0	213	0	293	0	0	0	0	0	29	0	5
	+	+	+	+	+	3	311	0	356	328	3	0	0	101	19	15	6
	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0

Results represent average colony numbers of three experiments, each one corresponding to a different isolate of bone marrow CD133⁺ cells. Cells were harvested after 14 days of culture with G-CSF and EPO, respectively, and sorted by CD13⁺ and CD36⁺ expression. Sorted cells were grown in semi-solid culture at a concentration of 40,000 cells /1.1mL. Supplemented with the indicated cytokine combination. Numbers in parentheses indicate sorted cell numbers.

* These numbers have been raftified from 8×10³ (CD13⁻CD36⁺) and 2×10³ (CD13⁻CD36⁻) cells in G14, and from 1.5×10⁴, 1×10⁴, 3.5×10⁴, and 1×10³ cells in E14, respectively. Colonies were scored on day 12~14. E, erythroid colonies; G, granulocyte colonies; M, monocyte colonies; E/G, mixed erythroid-granulocyte colonies.