A novel Lin-CD34+CD38-integrin α2- bipotential megakaryocyte-erythrocyte progenitor population in human bone marrow.

Wong, Wan Man; Dolinska, M; Sigvardsson, Mikael; Ekblom, Marja; Qian, H

Published in:
Leukemia

DOI:
10.1038/leu.2015.300

2015

OPEN

Leukemia (2015), 1–4

www.nature.com/leu

LETTER TO THE EDITOR

A novel Lin−CD34+CD38− integrin α2− bipotential megakaryocyte–erythrocyte progenitor population in the human bone marrow

Leukemia advance online publication, 27 November 2015; doi:10.1038/leu.2015.300

Haematopoiesis is a highly organized and complex developmental process where the hematopoietic stem cells lie at the apex of hierarchy and differentiate into all types of lineage-committed progenitors and mature blood cells. Identifying the distinct lineage-primed progenitors and gaining insights into the lineage commitment events are important for dissecting the developmental pathways of normal haematopoiesis and for understanding the underlying mechanisms in leukemogenesis.

In the adult human bone marrow (BM), megakaryocyte–erythrocyte progenitors (MEPs) have been identified in the committed progenitor enriched lineage-marker negative (Lin−) CD34+CD38+ compartment as CD123−CD45RA−, while common myeloid progenitors and granulocyte-macrophage progenitors have been defined as CD123^{lo}CD45RA− and CD123^{lo}CD45RA+,

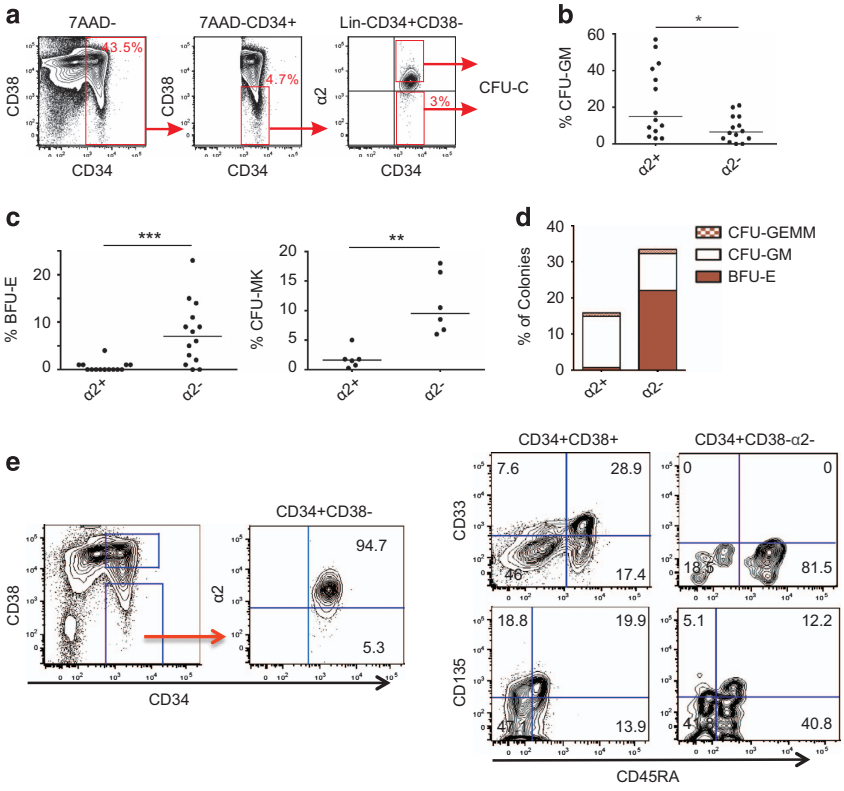

Figure 1. Erythroid and megakaryocyte colony-forming cells are enriched in human adult BM CD34+CD38− integrin α2− cell fraction. (**a**) Representative FACS profiles showing gating strategy for the isolation of CD34+CD38− integrin α2+ and integrin α2− cells. The numbers in the plots represent the percentage of cells in the gates. (**b**, **c**) The frequencies of granulocyte-macrophage (CFU-GM), erythroid (BFU-E) and megakaryocyte (CFU-MK) progenitors in CD34+CD38− integrin α2+ and integrin α2− cells assayed in the methylcellulose culture supplemented with cytokines (CFU-GM, BFU-E) or in collagen-based megakaryocyte colony-forming assay (CFU-MK). The horizontal bars show median values. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The results were obtained from two independent experiments with the use of 3–4 human samples. (**d**) Single-cell assay showing the frequencies of multipotent (CFU-GEMM), CFU-GM and BFU-E progenitors in Lin−CD34+CD38− integrin α2+ and integrin α2− cell fractions. The results are from 460 single Lin−CD34+CD38− integrin α2+ and 255 single Lin−CD34+ CD38− integrin α2− cells cultured in 96-well plates. The result was obtained from four independent experiments with six separate human samples in total. (**e**) Representative FACS profiles showing the expression of CD33 and CD135 in Lin−CD34+CD38+ progenitor cell-enriched cell fraction and Lin−CD34+CD38− integrin α2− cells gated for CD45RA expression. Shown are representative FACS plots of two separate experiments. Quadrant gates in the FACS plots were set on the basis of fluorescence minus one staining profiles by using isotype-matched monoclonal antibodies. The numbers in the plots represent the percentage of cells in the gates. Viable cells were gated by exclusion of 7AAD+ dead cells. 7AAD, 7-aminoactinomycin D.

Accepted article preview online 26 October 2015

respectively.[1] However, *in vitro* single cell cultures have shown that the more primitive stem cell-enriched BM Lin−CD34+CD38− fraction also contains progenitors of varying maturation and differentiation potentials, including MEPs.[2] So far, the markers to identify this distinct MEP population in the Lin−CD34+CD38− fraction have not been identified.

Here we show that the CD34+CD38− integrin α2− cell fraction of adult human BM contains in a high frequency hematopoietic progenitors with erythroid (burst-forming unit-erythroid (BFU-E)) as well as megakaryocytic (colony-forming unit-megakaryocyte (CFU-MK)) commitment analyzed in methylcellulose and collagen cultures, respectively. By using a single-cell serum-free liquid culture assay, we show that the Lin−CD34+CD38− integrin α2− cell fraction, further defined as CD45RA−, contains, in addition to unilineage-committed erythroid and megakaryocytic progenitors, in a high frequency MEP cells with bipotential differentiation capacity towards both lineages, enabling further studies on the molecular pathways regulating early erythroid–megakaryocytic commitment.

BM aspirates from healthy adult volunteers were collected with informed consent in accordance with institutional guidelines and approved by the local research ethics committee. Detailed information about the methods, including cell isolation, the antibodies used, colony assays and single-cell serum-free liquid cultures and gene expression analysis are described in Supplementary Information.

By fluorescence-activated cell sorting (FACS), integrin α2 was found to be expressed in most BM CD34+CD38− cells (96.7 ± 3.2%; mean ± s.d., $n = 25$; Supplementary Figure S1a), in contrast to a much more restricted expression in cord blood CD34−CD38− cells.[3] The CD34+CD38− integrin α2+ and integrin α2− cells were sorted by FACS for testing their colony-forming capacity (Figure 1a). The long-term culture-initiating cell assay, which measures hematopoietic stem cell activity but not committed progenitors, showed that

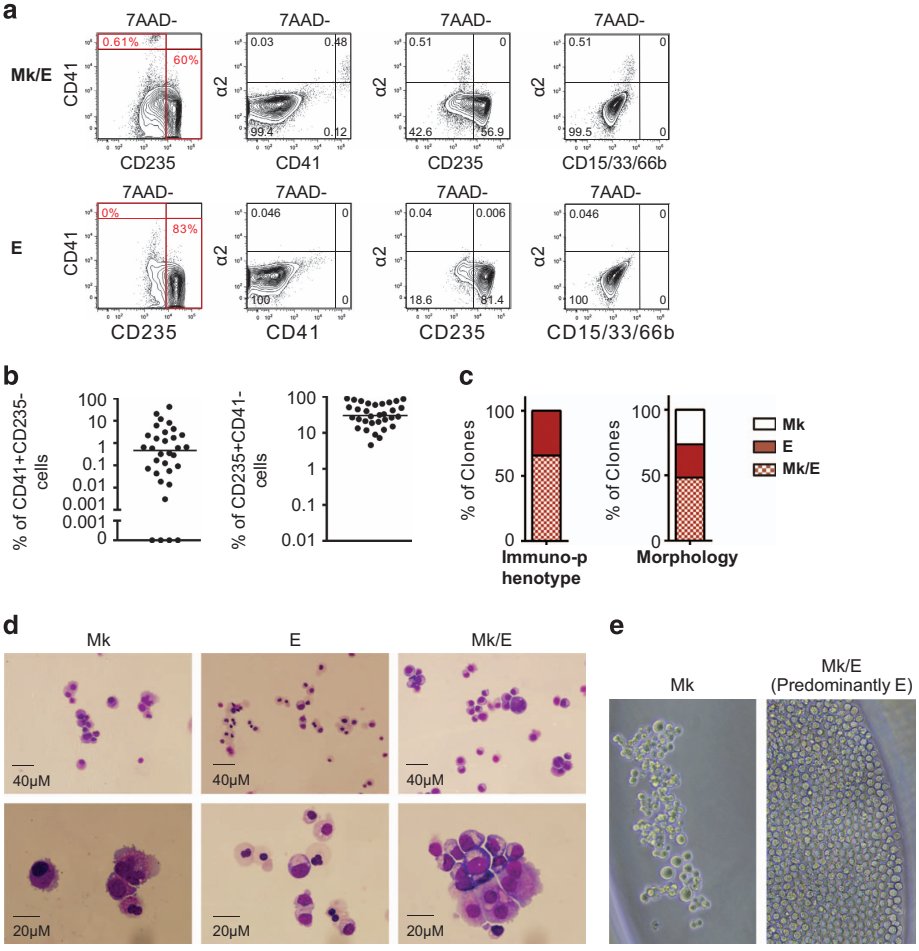

Figure 2. Single-cell clonogenic liquid culture assay reveals a high frequency of bipotent megakaryocyte–erythrocyte progenitors in human adult BM Lin−CD34+CD38−CD45RA− integrin α2− fraction. (**a**) Representative FACS profiles showing the expression of CD41, CD235 and CD15/CD33/CD66b in the individual clones derived from single Lin−CD34+CD38−CD45RA− integrin α2− cells after 12–14 days of serum-free culture with cytokines. Shown are clones derived from cells with bipotential megakaryocytic–erythroid (Mk/E) and unipotential erythroid (E) differentiation capacity. Quadrant gates in the FACS plots were set on the basis of fluorescence minus one control staining profiles using isotype control antibodies. The numbers in the plots show the percentage of cells in the gates. 7AAD−, viable cells gated by exclusion of 7AAD+ dead cells. (**b**) The frequency of cells showing megakaryocyte (CD41+CD235−) or erythrocyte (CD235+CD41−) differentiation determined by the FACS analysis of individual clones derived from single Lin−CD34+CD38−CD45RA− integrin α2− cells after 12–14 days of culture. Thirty-two individual clones were analyzed by FACS. (**c**) Lineage distribution of clones derived from single Lin−CD34+CD38−CD45RA− integrin α2− adult BM cells, evaluated by immunophenotype (FACS analysis) or by morphology on cytospin slides after May-Grünwald–Giemsa staining. (**d**) Morphology of the megakaryocyte (Mk), erythroid (E) and mixed megakaryocyte-erythroid (Mk/E) colonies derived from single Lin−CD34+CD38−CD45RA− integrin α2− cells. The cells were transferred onto cytospin slides and stained with May-Grünwald–Giemsa. (**e**) Morphology of the megakaryocyte (Mk), and mixed megakaryocyte–erythroid (Mk/E) colonies, derived from single Lin−CD34+CD38−CD45RA− integrin α2− cells, on Terasaki plates. The results were obtained from five independent experiments, each with one BM sample. 7AAD, 7-aminoactinomycin D.

long-term culture-initiating cells resided exclusively in the CD34+CD38− integrin α2+ fraction (Supplementary Figure S1b). The granulocyte-macrophage progenitors (CFU-GM) were significantly enriched in the integrin α2+ fraction (Figure 1b). Notably, the erythroid BFU-E and megakaryocyte CFU-MK progenitors were found in a significantly higher frequency in the CD34+CD38− integrin α2− than in the integrin α2+ fraction (Figure 1c). In agreement with these findings, single-cell colony assays showed that 22% of the single Lin−CD34+CD38− integrin α2− cells formed BFU-E, in contrast to $<1\%$ of the corresponding integrin α2+ fraction (Figure 1d). The CD45RA+ fraction within the Lin−CD34+CD38− integrin α2− cells had a very low myeloid clonogenic potential (data not shown), in line with a previous report,[4] and was excluded from further assays. The integrin α2+ and integrin α2− expressing cells within the Lin−CD34+CD38− compartment are not well demarcated, and therefore the minor CFU-GM population in the CD34+CD38− integrin α2− fraction in the methylcellulose cultures may represent contamination of the integrin α2+ cells.

CD33, a pan-myeloid marker, is expressed in granulocyte-macrophage progenitors and common myeloid progenitors but not in MEPs within the BM CD34+CD38+ cells.[5] Accordingly, the Lin−CD34+CD38−CD45RA− integrin α2− cell population was uniformly CD33− (Figure 1e). Within the Lin−CD34+CD38+ progenitor cell fraction, MEPs (CD123−CD45RA−) are predominantly CD135−, whereas most common myeloid progenitor ($CD123^{lo}$CD45RA−) are CD135+.[6,7] In agreement with this, our data show that most Lin−CD34+CD38−CD45RA− integrin α2− cells do not express CD135 (Figure 1e), providing additional evidence for the downregulation of CD135 during early erythroid and megakaryocytic differentiation.

The existence of bipotent MEPs in the Lin−CD34+CD38−CD45RA− integrin α2− cell fraction was studied by a single-cell liquid culture assay using serum-free culture conditions[2] (Figure 2). At days 12–14 of culture, 30–40% of the wells contained clones, with 1/3 of the clones covering 50–100% of the well surface area in Terasaki plates (Supplementary Figure S2b). Importantly, in FACS analysis of 32 clones derived from single Lin−CD34+CD38−CD45RA− integrin α2− cells, all clones contained CD41−CD235+ erythroid cells, whereas 22/32 of the clones contained both erythroid and megakaryocytic CD41+CD235− cells, confirming bilineage potential of these cells (Figures 2a and c). As shown previously,[2] the frequency of megakaryocytic cells was much lower than that of erythroid cells in the clones with bilineage differentiation (Figure 2b). During megakaryocytic differentiation, the cells acquired integrin α2 expression,[8] while the erythroid cells remained integrin α2− (Figure 2a).

For morphological analysis, 83 single-cell-derived clones were individually transferred to cytospin slides and stained by May-Grünwald–Giemsa (Figures 2d and e). This also allowed an evaluation of the clones with very low cell numbers, which could not be analyzed by FACS. These small clones invariably consisted of megakaryocytic cells (Figure 2e). Apart from these small megakaryocytic clones, bipotential MEP clones were identified by morphology in a similar proportion as by FACS analysis (Figure 2c). Taken together, our data demonstrate a high frequency of bipotential MEPs in adult BM Lin−CD34+CD38−CD45RA− integrin α2− cell fraction.

The cytokine combination used in the single-cell serum-free liquid cultures has been previously shown to support growth of myeloid progenitors.[2] In agreement with this, the clones derived from the single adult BM Lin−CD34+CD38−CD45RA− integrin α2+ cells cultured in the same serum-free liquid culture conditions generated 0.2–56% of myeloid cells (Supplementary Figure S3). In contrast, a low frequency of myeloid cells (0.13, 0.2 and 0.41% of cells) was seen in only 3 of the 32 Lin−CD34+CD38−CD45RA− integrin α2− single-cell-derived clones (data not shown), further supporting the notion that most of the cells are bipotent MEPs or their downstream progenitors.

Furthermore, our gene expression analysis provides molecular evidence for enrichment of erythroid and megakaryocytic-primed progenitors in the Lin−CD34+CD38− integrin α2− cells. Increased expression of hemoglobin delta was detected in integrin α2− cells (Supplementary Figure S4a, Supplementary Table S1). Notably, quantitative PCR analysis showed upregulation of the key regulator GATA-2, which is highly expressed in early erythroid cells and megakaryocytes,[9] and downregulation of hematopoietic stem cell regulator GATA-3[10,11] in the integrin α2− cell fraction (Supplementary Figure S4b), providing evidence that most integrin α2− cells are transcriptionally primed towards erythroid and megakaryocytic lineages

In conclusion, here we have identified a novel bipotential MEP cell population in Lin−CD34+CD38−CD45RA− integrin α2− cells in adult human BM. The identification of this novel MEP population provides a means for further analysis of the regulation of erythroid and megakaryocytic lineage fate decisions under physiological condition and for study of cellular mechanisms for myeloid leukemia. In addition, this finding may facilitate studies aiming to expand these lineages for clinical transfusion therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by research funding from Swedish Cancer Society (CAN 2010/590), Swedish Research Council (2008-19616-59131-19), Medical Faculty, Lund University, Skåne University Hospital, Gunnar Nilsson Foundation, John Persson Foundation, Gunnel Björk Foundation and Georg Danielsson Foundation to ME and Swedish Research Council (K2008-77PK-20879-01-2), Swedish Cancer Society (CAN 2009/1589, CAN 2012/891) and Karolinska Institute Wallenberg Institute for Regenerative Medicine to HQ. HQ is an assistant professor financed by Swedish Childhood Cancer Foundation (Barncancerfonden, FoAss13/015).

AUTHOR CONTRIBUTIONS

All the authors have participated in conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing and final approval of the manuscript.

WM Wong[1], M Dolinska[2], M Sigvardsson[3], M Ekblom[1,4,5] and H Qian[2,5]
[1]*Hematopoietic Stem Cell Laboratory, Department of Laboratory Medicine, Lund University, Lund, Sweden;*
[2]*Center for Hematology and Regenerative Medicine, Department of Medicine, Karolinska Institute, Stockholm, Sweden;*
[3]*Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden and*
[4]*Department of Hematology and Coagulation, Skåne University Hospital, Lund, Sweden*
E-mail: hong.qian@ki.se
[5]*These authors contributed equally to this work*

REFERENCES

1 Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 2000; **404**: 193–197.
2 Debili N, Coulombel L, Croisille L, Katz A, Guichard J, Breton-Gorius J *et al.* Characterization of a bipotent erythro-megakaryocytic progenitor in human bone marrow. *Blood* 1996; **88**: 1284–1296.
3 Wong WM, Sigvardsson M, Astrand-Grundstrom I, Hogge D, Larsson J, Qian H *et al.* Expression of integrin alpha2 receptor in human cord blood CD34+CD38-CD90+ stem cells engrafting long-term in NOD/SCID-IL2Rgamma(c) null mice. *Stem Cells* 2013; **31**: 360–371.
4 Doulatov S, Notta F, Eppert K, Nguyen LT, Ohashi PS, Dick JE. Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. *Nat Immunol* 2010; **11**: 585–593.

5 Taussig DC, Pearce DJ, Simpson C, Rohatiner AZ, Lister TA, Kelly G *et al.* Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. *Blood* 2005; **106**: 4086–4092.
6 Kikushige Y, Yoshimoto G, Miyamoto T, Iino T, Mori Y, Iwasaki H *et al.* Human Flt3 is expressed at the hematopoietic stem cell and the granulocyte/macrophage progenitor stages to maintain cell survival. *J Immunol* 2008; **180**: 7358–7367.
7 Edvardsson L, Dykes J, Olofsson T. Isolation and characterization of human myeloid progenitor populations--TpoR as discriminator between common myeloid and megakaryocyte/erythroid progenitors. *Exp Hematol* 2006; **34**: 599–609.
8 Nieswandt B, Brakebusch C, Bergmeier W, Schulte V, Bouvard D, Mokhtari-Nejad R *et al.* Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. *EMBO J* 2001; **20**: 2120–2130.
9 Anguita E, Hughes J, Heyworth C, Blobel GA, Wood WG, Higgs DR. Globin gene activation during haemopoiesis is driven by protein complexes nucleated by GATA-1 and GATA-2. *EMBO J* 2004; **23**: 2841–2852.
10 Georgantas RW 3rd, Tanadve V, Malehorn M, Heimfeld S, Chen C, Carr L *et al.* Microarray and serial analysis of gene expression analyses identify known and novel transcripts overexpressed in hematopoietic stem cells. *Cancer Res* 2004; **64**: 4434–4441.
11 Buza-Vidas N, Duarte S, Luc S, Bouriez-Jones T, Woll PS, Jacobsen SE. GATA3 is redundant for maintenance and self-renewal of hematopoietic stem cells. *Blood* 2011; **118**: 1291–1293.

Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)