



REVIEW

AC133 expression in human stem cells

M Bhatia

The John P Robarts Research Institute, Developmental Stem Cell Biology, The University of Western Ontario, Ontario, Canada

Expression of cell surface markers on human hematopoietic cells has provided a method for characterizing subsets of cells with distinct biological functions. This is largely due to the ability to separate highly purified subpopulations of cells for comparative analysis. Relationships between the cell surface phenotype of these subpopulations and their proliferative and differentiative capacity have been instrumental in defining the hierarchical organization of cells comprising the human hematopoietic system. The identification and isolation of human hematopoietic cells expressing AC133, combined with use of *in vitro* and *in vivo* assays, has provided novel insights into the hematopoietic progenitor and stem cell compartment in the human. More recent studies have offered evidence that AC133 expression is not limited to primitive blood cells, but also defines unique cell populations in non-hematopoietic tissues. These findings will be reviewed here in the context of human hematopoiesis and the potential role and utility of AC133 expression in the human. *Leukemia* (2001) 15, 1685–1688.

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AC133: a novel 5 transmembrane protein

AC133 is thought to best represent the human homolog of prominin 5-transmembrane glycoproteins (PROML 1).^{1–3} Although murine prominin was originally identified as a protein expressed on murine neuroepithelial cells, it has recently been shown to be phylogenetically conserved from mammals to zebrafish, *Drosophila* and *Caenorhabditis elegans*, and expressed in a wide variety of regions in these organisms.^{4,5} The specific function and potential ligands for the prominin family remain to be characterized, however, human and mouse versions of prominin have been shown to share similar selective membrane association and tissue expression profiles.^{5,6} In the human, monoclonal antibodies to AC133 have been described that may bind different epitopes, and were originally shown to react with cell surface antigens expressed on primitive hematopoietic progenitors.^{2,7} Together with other cell surface markers associated with primitive blood cells, these antibodies provided a means for subsequent analysis and isolation of AC133 expressing and non-expressing cells.

AC133 expression: cell phenotype and biological function

The majority of cells reactive to the AC133 antibodies were found to co-express the human stem/progenitor marker CD34.⁷ Human subpopulations of AC133⁺ cells are present throughout human hematopoietic ontogeny,^{8,9} although the proportions of subpopulations differ throughout human devel-

opment.⁹ Aside from these ontogenic differences, AC133⁺ cells are present in the peripheral circulation of mobilized and non-mobilized adults, and in the bone marrow compartment.^{10–13} Several investigators have demonstrated the presence of AC133⁺ cells that co-express CD34, c-kit, and other cell surface markers,^{13–16} and the majority of these studies conclude that AC133 provides an important marker for identification of primitive progenitors and stem cell populations upon *de novo* isolation. Isolation and *in vitro* culture of AC133⁺ cells indicate that functional hematopoietic progenitors can be expanded from these selected cells in a similar manner to that of CD34 or c-kit populations,^{12,13,17,18} thereby suggesting that AC133⁺ cells share similar growth factor requirements. However, the use of antisense approaches¹⁹ suggest that AC133 may not be required for proliferation and differentiation of human progenitors¹⁴ and the functional relationship of AC133 may be inconsequential to primitive stem/progenitor properties. Taken together, these studies clearly indicate that AC133 represents a significant cell surface marker for identification of human progenitor/stem cells, but it remains unclear whether use of this marker provides any distinct advantage over CD34⁺ blood cells isolation or cellular expansion.

Purging of transformed human hematopoietic clones has been a major goal for the treatment of a variety of childhood and adult leukemias for autologous transplantation.^{20,21} However, cell surface marker expression to identify residual normal stem/progenitor cells in leukemic patients has been limited to more commonly used markers such as CD34, c-kit, and Thy antigen expression.^{21–24} AC133⁺ cells have been shown to be expressed in both acute^{16,25–27} and chronic myeloid leukemias²⁸ and lymphoblastic leukemias,^{29,30} of both adult and pediatric patients.³¹ To date, these combined studies indicate that the AC133 antigen expression is related to CD34 cell surface expression and AC133 may provide alternative, but similar information with regards to leukemic blast phenotype in acute myeloid leukemia (AML)s.^{25,31} However, in the case of lymphoblastic leukemia, encouraging preliminary observations suggest that AC133 may provide an important marker capable of distinguishing normal stem/progenitors from lymphoid leukemia initiating blasts. Isolation and transplantation of highly purified subsets of AC133⁺ and AC133⁻ cells from both AML and acute lymphoblastic leukemia (ALL) patients into *in vivo* models (such as immune-deficient recipient mice) capable of detecting human leukemic stem cells³² will be required to determine the potential benefits of AC133 in purging of leukemic cells for autologous transplantation.

Novel subpopulations of AC133⁺ cells

In addition to hematopoiesis, AC133 expressing cells have been shown to possess endothelial capacity. In a series of elegant studies, Rafii's group has identified a population of

Correspondence: M Bhatia, The John P Robarts Research Institute, Developmental Stem Cell Biology, 100 Perth Drive, London, Ontario, N6A 5K8, Canada; Fax: (519) 663-3789
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human AC133⁺ cells that co-express vascular endothelial growth factor receptor (VEGF-R) and CD34 using *in vitro* and *in vivo* human-mouse xenograft assays.³³ Furthermore, the same group has characterized a functional role for these cells during vascular trauma and suggest these circulating endothelial progenitors may be mobilized by chemokines such as VEGF, and in turn may be useful in accelerating repair of injured vascular tissue.³⁴

In human hematopoietic tissue, discordant expression of AC133 and CD34 has been demonstrated.^{8,15,35} The presence of AC133⁺CD34⁻ cells was reported within a variety of hematopoietic tissues in both adult and cord blood (CB) sources. Using full-term human CB, our group has used AC133 to identify a unique subpopulation of CD34⁻ cells with primitive stem/progenitor cell properties.⁸ These human hematopoietic cells are devoid of classically defined stem cell-associated surface markers and respond in a distinct manner to *ex vivo* culture in comparison to CD34⁺ cells.^{8,36} Their distinguishing feature is the undetectable expression of CD34, HLA-DR, and Thy-1 cell surface markers, and they reside in a highly purified fraction of cells that are depleted of lineage commitment markers (Lin⁻). In addition to these phenotypic differences, we originally described several lines of evidence that functionally distinguished CD34⁻CD38⁻Lin⁻ cells from CD34⁺ cells.³⁶ Although the majority of CD34⁻CD38⁻Lin⁻ cells lack AC133 and express a lymphoid-associated marker CD7,^{8,37} an extremely rare population of AC133⁺CD7⁻ cells was identified at a frequency of less than 0.02%.⁸ Surprisingly, these AC133⁺CD7⁻ cells were highly enriched for progenitor activity at a frequency equivalent to purified fractions of CD34⁺ cells,⁸ and were the only subset amongst the CD34⁻CD38⁻Lin⁻ population capable of giving rise to CD34⁺ cells in defined liquid cultures.⁸ Human cells were detected in the bone marrow of NOD/SCID mice 8 weeks after transplantation of *ex vivo* cultured AC133⁺CD7⁻ cells isolated from the CD34⁻CD38⁻Lin⁻ population, whereas 400-fold greater numbers of the AC133⁻CD7⁻ subset had no engraftment ability.⁸

More recently, our laboratory has shown the presence of AC133⁺CD34⁻Lin⁻ cells in G-CSF and G-CSF + SCF-mobilized peripheral blood cells in adults. These AC133⁺CD34⁻ cells are capable of similar progenitor capacity and differentiation potential into CD34⁺ cells as CB-derived cells.³⁸ In contrast to CD34⁻Lin⁻ cells devoid of AC133 expression, AC133⁺CD34⁻ cells can be transduced by gibbon ape leukemia virus-receptor (GALV-R) pseudotyped retrovirus at a similar efficiency as highly purified subsets of CD34⁺ cells.³⁹ Therefore, AC133 expression has provided novel insights into the hierarchical relationship of the human stem cell compartment by identifying a population of primitive human CD34⁻ negative stem cells that is also enriched for progenitor function and is capable of producing CD34⁺ cells. Based on the findings of our group and others, we propose a working model shown in Figure 1, that illustrates the potential relationships of cellular subsets derived from human hematopoietic tissue. We propose that CD34⁻CD38⁻Lin⁻ cells give rise to CD34⁺ subsets with hematopoietic⁸ and endothelial capacity,³³ but this function is only found with the AC133⁺ subfraction. AC133⁻ cells within the CD34⁻CD38⁻Lin⁻ fraction contain cells termed side population (Sp) due to their dye efflux properties, but the majority co-express CD7 and have been suggested to represent lymphoid restricted progenitors.³⁷ The extent to which these AC133⁺CD34⁻Lin⁻ cells participate in reconstitution and hematological recovery upon transplan-

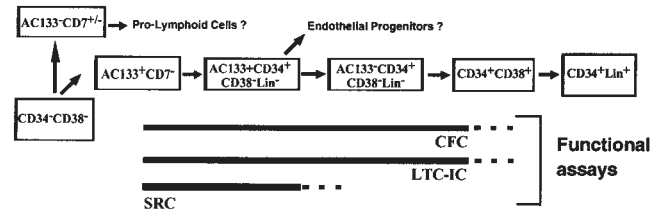


Figure 1 Proposed working model of the organization of human hematopoietic stem/progenitor cells based on phenotypic subsets of cells detected using *in vitro* and *in vivo* assays.

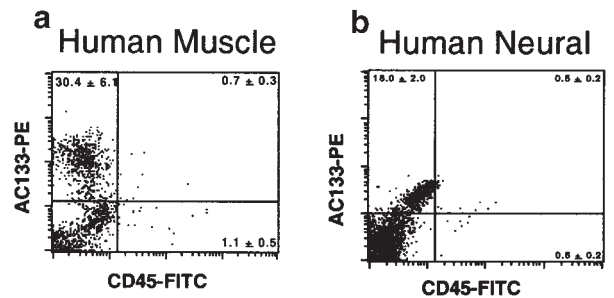


Figure 2 Expression of AC133 (CD133) on human skeletal muscle (a) and neural cells (b). The anti-AC133 antibody was purchased from Miltenyi Biotec, Auburn, CA, USA. The majority of AC133⁺ cells identified are devoid of the human hematopoietic marker CD45.

tation await clinical marking studies and analysis of diagnostic correlates during the transplantation procedure.

Future perspectives

AC133 may provide an important marker for hematopoietic stem and progenitor cells in the human, but its clinical relevance and potential benefit over and above available cell surface markers such as CD34, and c-kit or Thy-1 remain to be determined. However, the utility of AC133 for stem cell purification may not be restricted to hematopoietic tissue. Our laboratory has found that AC133 is expressed on a subset of cells within both skeletal muscle and human neural tissue, the majority of which is also devoid of the hematopoietic commitment marker CD45 (Figure 2a and b). The more global expression of AC133 and its relationship to human stem cell capacity is further supported by recent reports that demonstrate AC133 selection enriches for human neural cells capable of reconstituting the brain of neonatal immune-deficient mice⁴⁰ and the detection of AC133 transcripts in embryoid bodies derived from human embryonic stem cells.⁴¹ These preliminary observations indicate that the functional significance of AC133 transcends hematopoietic tissue and in fact may represent a marker for a variety of uncommitted human stem/progenitor cells.

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