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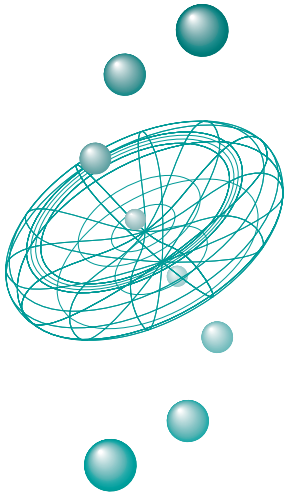

SERIES
14

CD34 Negative Hematopoietic Stem Cells

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INTRODUCTION

Two of the most exciting breakthroughs in hematopoietic stem cell (HSC) research were first, the discovery of CD34 expression on HSC, anti-CD34 antibody development and its applications in HSC transplantation[1, 2]; and secondly, the recent discovery of CD34 negative (CD34⁻) HSC[3-10]. These discoveries, together with the success in studies of other stem cells, have resulted in the emergence of novel clinical treatments in stem cell regenerative medicine.

Following the studies of CD34 by Civin[1] in 1988, CD34 antibody selected cells were successfully used for the reconstruction of hematopoiesis in lethally irradiated baboons[2]. Since then, the CD34 marker and antibody have been widely used in the research of hematopoietic stem cell biology and clinical medicine, because of the effectiveness of anti-CD34 antibody selected cells in hematopoietic reconstitution for both animal investigation and human transplantation. These findings originally established the belief that hematopoietic stem cells are CD34 positive (CD34⁺). This is why the first report about CD34⁻ HSC and its function in long-term lympho-hematopoietic reconstitution by Osawa, and other consequent studies[3-10], have significantly challenged the existing concepts in stem cell biology and related clinical applications, such as stem cell transplantation and gene therapy[4]. Therefore, it is not surprising that many HSC investigators have used some interesting titles for their papers, and commonly question marks have appeared in the article titles.

For example:

- CD34⁻ stem cells as the earliest precursors of hematopoietic progeny[5]
- Hematopoietic stem cells: Are they CD34-positive or CD34-negative?[6]
- CD34⁺ or CD34⁻: Does it really Matter? [7]
- Who is hematopoietic stem cell: CD34⁺ or CD34⁻? [8]
- CD34: To select or not to select? That is the question [9]
- Development of the hematopoietic stem cell: Can we describe it? [10]

Imaginably, these question marks also exist in the areas of HSC biology, its clinical applications, and future directions in stem cell investigations. This review will briefly summarize the recent progress in CD34⁻ HSC research in basic and clinical medicine including CD34⁻ HSC biology, clinical applications, expansion, and detection.

BIOLOGY OF CD34 NEGATIVE HSC

Relationship to CD34$^+$ cells

Because of the history discussed above, and the close relationship between CD34$^+$ and CD34$^-$ HSCs, the developing stages and biological role of CD34$^-$ HSC are crucial to our better understanding of applying stem cells in clinical treatment. Huss[5] discussed and illustrated this relationship. Similar illustrations have been published in other articles[7, 11]. Bonnet further illustrated the current understanding of the relationship between CD34$^+$ and CD34$^-$ cells in both mouse and human[12]. The adherent CD34$^-$ HSC possesses a capability for self-renewal, and can differentiate into CD34$^+$ HSC that can change back to CD34$^-$ HSC again in some circumstances. This cycle, including the CD34$^-$ HSC pool and CD34$^+$ HSC pool, was named the "stem cell cycle". The cells in the cycle are partially in bone marrow and partially in peripheral blood. It is now becoming more apparent that the earliest hematopoietic stem cell population is CD34$^-$, but these cells can differentiate into CD34$^+$, circulating in the blood, homing back to bone marrow, and repopulating progenitor cells[5, 11]. The relationship between CD34$^-$, CD34$^+$ and other blood cells can be summarized in ***Fig. 1*** according to current investigations.

Different methods were adapted to study the relationship. *Ex vivo* experiments indicated the generation of CD34$^+$ cells from CD34$^-$ HSC. The cultured CD34$^-$ Lin$^-$ cells acquired colony-forming ability and turned into CD34$^+$ cells, further confirming that the CD34$^-$ HSC is at an earlier stage than the CD34$^+$ HSC[11, 13-16]. An investigation using human/sheep competitive engraftment models on the *in vivo* engraftment potential of human CD34$^-$ Lin$^-$ cells has also indicated that the CD34$^-$ fraction of normal human bone marrow contains cells capable of engraftment and differentiation into CD34$^+$ progenitors and multiple lymphohematopoietic lineages in primary and secondary hosts[14]. Further investigation has established a concept of reversible expression of CD34 on the HSC[5, 11, 17-19]. The mechanisms of reversible CD34 expression, HSC differentiation, proliferation, and return to a state of "quiescence" are mediated by cell-cell interactions and growth factors produced mainly by the cells of the marrow microenvironment, or by the fibroblast-like CD34$^-$ stem cells themselves. The major factor to induce differentiation and have CD34$^-$ transit to CD34$^+$ is stem cell factor (SCF; c-kit ligand). Interleukin-6 rather promotes proliferation and maintains the adherent growth as CD34$^-$ cells. The related cell cycle studies indicate that SCF induces the expression of the cyclin-dependent kinase inhibitor p27^{kip-1} that blocks proliferation during differentiation. IL-6 completely suppresses p27 expression, enabling hematopoietic stem cell to proliferate[5]. The animal investigation suggests at least some of these CD34$^-$ stem cells convert to a CD34 phenotype upon activation, and after transplantation, the CD34$^+$ stem cells revert to a CD34$^-$ phenotype. Therefore, CD34 may be a marker of activated stem cells, but not necessarily all stem cells[7].

Biological potential of CD34$^-$ stem cells

The primary ability of these cells is to maintain and reconstitute the hematopoietic system because they are the earliest HSCs and can develop into CD34$^+$ cells, whose capability in hematopoiesis has been clearly indicated. Furthermore, the perspective to use CD34$^-$ stem cells in clinical practice may become much broader in the future according to recent research in stem cell biology. Bone marrow derived mesenchymal cells have shown ability in the formation of osteocytes, hepatic cells and cardiomyocytes. The latest research shows that the potential of CD34$^-$ stem cells is almost unlimited in its ability to generate whole organ systems. The isolation and expansion of CD34$^-$ HSCs may provide more interesting research in stem cell biology and important clinical applications[11].

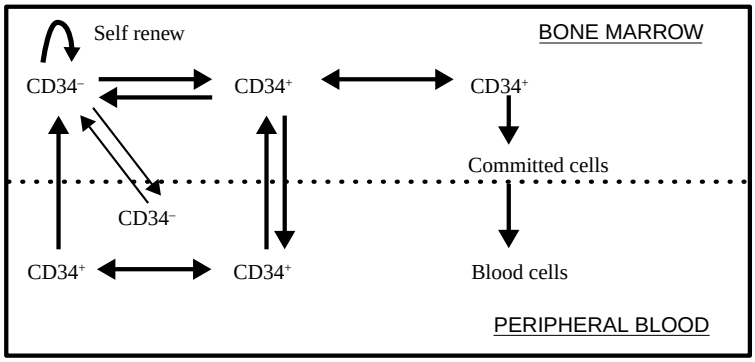

Fig. 1 *The relationship between CD34$^-$, CD34$^+$ HSC, and other blood cells in human bone marrow and peripheral blood according to current understanding.*

Studies in animal and human

Murine HSCs have been commonly used in human HSC investigations, since murine hematopoiesis has been recognized as a good model for human hematopoiesis and the basic principles regulating murine stem cells appear to apply to human stem cells[4]. The data from several different species and animal models of hematopoietic stem cell functions indicate that the hematopoietic stem cell compartment contains more than one phenotypically identifiable population capable of self-renewal and long term pluripotent engraftment. It is clear that some stem cells express CD34, and others do not[4, 17,18]. The animal experimentation has also indicated the change of CD34 expression at different development stages[19-22]. The studies with murine models indicate that normal adult mice stem cells are CD34 negative in the steady state bone marrow[3, 17, 20-22]. Further observation indicates that all stem cells from neonatal to 5-week old mice are $CD34^+$. In 7-week old mice $CD34^-$ stem cells begin to emerge. The majority of the stem cells in the 10- and 20-week old mice are $CD34^-$ [4, 19, 20]. Based on the results from animal testing, human cells have also been investigated. One special group of cells referred to as "side population" (SP) was found in human and rhesus bone marrow. The SP are largely $CD34^-$ /low. After 5 weeks of suspension culture on bone marrow stromal cells, the rhesus and human $CD34^-$ SP cells became $CD34^+$. Long- term engrafting cells also reside in the $CD34^-$ population[16, 17]. On the other hand, the transplantation studies showed that both the $CD34^+$ and $CD34^-$ populations of human cells are capable of long-term engraftment. It is also believed the adult human and murine stem cells are $CD38^-$ and $CD38^+$, respectively[4]. The human $CD133^+$ $CD34^-$ $CD38^-$ Lin^- cells are capable of acquiring CD34 and possess a colonogenic progenitor capacity equivalent to primitive $CD34^+$ cells[23]. These studies indicate the existence of $CD34^-$ HSCs in both animals and humans. However, because of the differences between animals and human beings, the animal investigations can only provide a prediction or hypothesis. Many unanswered questions are still waiting for data from research on human cells.

Other cell markers and expressions

Other cell markers are commonly used for HSC investigation to help judge HSC development and cell purity. The use of a combination of CD34 and other cell markers in mice bone marrow indicates that long-term engrafting cells in Lin^-c-Kit^+ $Sca\text{-}1^+$ bone marrow cells are $CD34^-$[4]. However, it was also considered that one possibility of elimination of some $CD34^+$ stem cells with the negative immunoaffinity method used for the preparing Lin^- cells was due to the expression of lineage-specific markers. CD38 expression on the human HSC was investigated. The CD38 expression by steady-stage and activated stem cells of juveniles is still unknown[4]. Other cell markers including AC133, CD7 and CD38[23, 24] have been used in human $CD34^-$ HSC selection[23, 24]. CD45 is expressed on all nucleated peripheral blood cells; it has been used to distinguish progeny of tested populations. Other lineage markers including CD4, CD14, CD19, CD45, CD2, CD3, CD16, CD41, CD56, and glycophorin A have been used in cell purification, and purity checking for HSCs[25].

Isolation of $CD34^-$ HSCs

Modern cell isolation techniques are essential to HSC investigation and clinical application. One of most difficult aspects of the characterization of human $CD34^-$ stem cells is the difficulty in detecting their function using *in vitro* assays. These cells only demonstrate hematopoietic activity *in vivo* and lack a marker for positive selection[6]. Various methods have been used for cell purification or enrichment of $CD34^-$ HSCs[23-25]. AC133 is a novel marker for human hematopoietic stem and progenitor cells. It was named CD133 at the 7th International Workshop and Conference on Human Leukocyte Differentiation Antigens, 2000[23, 26]. Gallacher used cell surface markers AC133 and CD7 to select cells from $CD34^-$ $CD38^-Lin^-$ cells and found that the majority of $CD34^-$ $CD38^-Lin^-$ cells lack AC133 and express CD7. However, interestingly, a very rare population of $AC133^+CD7^-$ cells was found with a frequency of 0.2% and high progenitor activity equated to the purified fractions of CD34 stem cells. These cells appear to be the ones that can change to $CD34^+$ cells in the defined liquid culture[23]. Nakamura used a newly developed filter system to enrich $lineage^-$ $CD34^-$ cells[25]. The frequency of Lin^- $CD34^-$ cells in the cell population after filtration reached 7.45 ± 4.41% with a 16.8 ± 8.81 fold enrichment. The mean recovery of Lin^- $CD34^-$ cells was 48.57 ± 13.59%. Therefore, the authors believe the method is useful for isolating Lin^- $CD34^-$ cells and can assist in the research of Lin^- $CD34^-$ cells. Other methods[27-30] have also been used in cell isolation. Kim[29] compared the hematopoietic activities of human bone marrow and umbilical cord blood CD34 positive and negative cells. Huss reviewed the isolation of primary and immortalized $CD34^-$ hematopoietic and mesenchymal stem cells from various sources that include bone marrow, peripheral blood, fetal liver, and umbilical cord blood. Negative selection with multi-antibodies including CD34 and Lin was also used for Lin^- $CD34^-$ cell selection from human umbilical cord blood[13]. This method showed that the frequency of Lin^- $CD34^-$ cells among all nucleated cord blood cells was 0.58% ± 0.36% (mean ± SD, n=11). The total number of collected Lin^- $CD34^-$ cells ranged from 0.4×10^5 to 3.7×10^5 (mean, $1.7\pm1.2\times10^5$). It is believed that $CD34^-$ stem cells are predominately part of the quiescent stem cell pool of hematopoietic and mesenchymal stem cell[30].

CLINICAL APPLICATION OF $CD34^-$ HSC

For more than one decade, $CD34^+$ HSC/HPC transplantation has been used in the reconstitution of hematopoiesis in patients suffering ablative treatment or for building up chimeric bone marrow without pre-intensive treatment[31]. The discovery of $CD34^-$ HSC has caused the emergence of valuable clinical applications, particularly in regenerative medicine and gene therapy, based on new insights regarding their characteristics of self-renewal, rapid differentiation, and multi-organ, multi-lineage engraftment[32-45].

Regenerative Medicine

HSCs have been used in regenerative medicine including hematopoietic regeneration by bone marrow, peripheral blood and cord blood $CD34^+$ cell transplantation. Recent research on HSC biology and other stem cells has suggested the role of $CD34^-$ HSC in multi-organ development because of its plasticity (which can be defined as the ability of an adult stem cell from one tissue to give rise to specialized cells or another tissue or organ), the cells can be used, not only in hematopoietic reconstitution, but also in other types of regenerative medicine[11].

Hematopoietic reconstitution

HSCs supply all blood cells throughout life through their self-renewal capabilities and multi-lineage differentiation. For over 10 years, bone marrow and peripheral blood $CD34^+$ cells have been routinely used in stem cell transplantation. Now, it is demonstrated that $CD34^-$ stem cells are able to reconstitute all hematopoietic lineages[30]. The "stem cell cycle" theory has provided further support for $CD34^-$ HSC transplantation.

The gold standard used to prove HSC function relies on the animal experimentation that shows the ability of those cells to reconstitute lethally irradiated animals. The discovery of the $CD34^-$ HSC has challenged the concept that $CD34^+$ cells are primary hematopoietic stem cells, and has stimulated novel discussions and research in HSC transplantation[46-50]. Osawa first demonstrated the ability of $CD34^{-/low}$ cells in lymphohematopoietic reconstitution in mice[3]. The long-term lymphohematopoietic reconstitution by a single $CD34^{-/low}$ HSC and other experiments indicates that the reconstitution function starts out as $CD34^-$ HSC. At least some of these $CD34^-$ stem cells convert to a $CD34^+$ phenotype upon activation. After transplantation, the $CD34^+$ cells can home to bone marrow and revert to a $CD34^-$ phenotype. The application of peripheral blood stem cells has significantly improved progress related to stem cell transplantation[51]. Huss has further described that the $CD34^-$ stem cells can also be isolated from human peripheral blood mononuclear cells[11]. The "stem cell cycle" model, and the concept of reversible CD34 expression of HSC has made it possible to use peripheral blood $CD34^-$ HSC in regenerative medicine[5, 11]. The sheep *in utero* transplantation of human hematopoietic stem cells has indicated the engraftment potential of human $CD34^-$ Lin^- bone marrow cells, and has shown the appearance of $CD34^+$ cells in animals transplanted with $CD34^-$ cells[6, 14]. A more important implication of this study is that *in utero* transplantation of HSC may have a potential role in the clinical treatment of different congenital diseases.

Other regenerative medicine[32-35, 44, 52-56]

The promising results from recent investigations suggest broadened indications for $CD34^-$ HSC in cellular and gene therapy. With the extended knowledge in the biology of hematopoietic and mesenchymal stem cells, new therapeutic strategies can be established. We are getting closer to generating whole organ systems by using single autologous blood cells for medical use. It was predicted that $CD34^-$ stem cells would provide a new tool in the treatment of diseases that affect the hematopoietic and mesenchymal organ systems. Depending on their stage of differentiation, $CD34^-$ stem cells can generate not only hematopoietic progenitors, but also more specified mesenchymal precursors, such as osteoblasts, chondrocytes, myocytes, adipocytes, and others. The latest developments show the potential of $CD34^-$ stem cells in generating whole organ systems. These developments may provide clinicians with many more opportunities in stem cell regenerative medicine. Huss believed that $CD34^-$ stem cells would certainly be valuable cellular tools in the near future for autologous organ replacement therapy as an example[11]. Recent research on the multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell has indicated the capability of engraftment of an HSC into different organs. The adult bone marrow cells have tremendous differentiating capacity as they can also differentiate into epithelial cells, liver, lung, gastrointestinal tract, and skin. This finding may contribute to clinical treatment of genetic disease or tissue repair[32-35]. A publication in March 2002 has further shown hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells and has concluded that circulating stem cells can differentiate into mature hepatocytes and epithelial cells of the skin and gastrointestinal tract[34]. It has also directly been shown that $CD34^-$ blood-derived cells readily differentiate into EC-like cells in cultures and they incorporate into the neovasculature *in vivo*, indicating that these cells have an angioblastic potential. This can be useful in angiogenesis treatment[33]. These studies indicate the role of $CD34^-$ HSC from different sources in regenerative medicine. With easier access to greater numbers of cells compared to embryonic stem cells, HSCs may potentially be used more and more for regenerative medicine in the treatment of various diseases. Embryonic stem cell studies have also further indicated pluripotency in multi-organ and multi-lineage differentiation[32, 56] based on the results from studies of HSC and other stem cells. Stem cell transplantation therapies have significantly extended their clinical applications in regenerative medicine[48, 56]. To improve clinical outcomes, some methods such as genetic manipulation of MHC genes, nuclear reprogramming, and hematopoietic chimera can be used to circumvent host immune-mediated rejections when embryonic stem cells are used for regenerative medicine[56].

Gene therapy

The HSC genetic program has been considerably investigated[57-59]. Efficient gene therapy needs to transfer functional genes into stem cells with characteristics of self-renewal, long-term hematopoiesis/survival, and differentiation into various types of mature cells. Marrow cells are currently the most widely utilized targets in human gene therapies. HSC may be the best target of all. Able to both self-replicate and engender blood cells in all lineages, they could be a lifelong source of therapeutic genes for disorders in any lineage. Gene transfer into HSCs could lead to a continuous supply of genetically modified hematopoietic cells for the lifetime of the recipient. The discovery of $CD34^-$ HSC may provide ideal cells to meet the requirements in retroviral gene transfer,

and lentiviral vector-mediated gene transduction[60, 61]. The cells used for lentiviral vector mediated transduction are $CD34^-$ /low $c\text{-}Kit^+Sca\text{-}1^+Lin^-$ ($CD34^-$ KSL). Lentiviral vectors can efficiently transduce genes into highly enriched murine HSCs and sustain a long-term expression of the trans-gene in the multilineage differentiated progeny in reconstituted mice. $CD34^-$ HSC was also isolated from peripheral blood cells, then cloned after immortalization with SV-40 large T-antigen containing retroviral vector[30, 58].

The highly efficient gene transfer into $CD34^-$ HSCs, and their organ-specific distribution and differentiation imply the use of $CD34^-$ HSC in cellular and gene therapy because the evidence that has emerged suggests that long-term hematopoietic reconstitution can be achieved solely with $CD34^-$ cells[60]. Even though there is still an ongoing discussion whether there is a common precursor cell of the marrow microenvironment and hematopoiesis[30], it is reasonable to consider that $CD34^-$ stem cells in bone marrow partially consist of the mesenchymal stem-cell pool, which has already been used to generate specific organ systems. Gene therapy by using the cells may be able to correct the defective function in those organs. Gene therapy with HSC may also portend the therapeutic use of even more versatile embryonic stem cells.

Other clinical applications

CD34 expression on normal and leukemia cells has been studied[12, 62-65]. In one study, 41 cases of acute leukemia were examined by using AC133, CD34 and other markers. All $AC133^+$ leukemias also expressed CD34. Thirteen of 33 $CD34^+$ leukemias were negative for AC133. No leukemia with $AC133^+$/ $CD34^-$ was found. The prognostic analysis demonstrates that AC133 expression in AML blasts is associated with poor clinical outcomes in terms of earlier relapse and shorter disease-free survival. This suggests that the AC133 antigen might provide the prognostic stratification of acute leukemia[62]. Another study[63] directly focused on the CD34 expression in adult acute myeloid leukemias and its prognostic significance. Fifty-one adult AML patients exhibited a hypercellular bone marrow with >75% blasts. Twenty-seven out of fifty-one AML patients (52.9%) were found to be $CD34^+$ (mean CD34 expression: 63.52 +/- 19 .66%) with a 20% cutoff, whereas twenty-four patients were $CD34^-$ (CD34 expression mean: 6.13 +/- 6.72%). AML patients who were $CD34^+$ had a lower complete remission (CR) rate (47.3%) as compared to AML patients who were $CD34^-$ (66.6%). The authors concluded that these results, although still limited, suggested the prognostic value of CD34 in AML patients, and showed how the CD34 antigen expression relates to the degree of blast cell differentiation. A report with 2,483 AML patients suggested that CD34 expression does not predict complete remission of AML. Twelve studies in the report showed significantly better complete remission rates in $CD34^-$ patients, whereas ten other studies failed to show a difference between $CD34^+$ and $CD34^-$ patients[64]. The observation on the "side population" suggested this population would be an intriguing candidate for a chemoresistant, relapse initiating cell in patients with AML[65]. The clinical research developed the theoretical question "does leukemic transformation occur at the $CD34^-$ stem cell level?" The questions "are leukemic Lin^- $CD34^-$ cells, like their normal counterparts, capable of inducing CD34 'activation'?" and "are leukemic $CD34^-$ and $CD34^+$ cells interconvertible?" should also be answered[12].

EXPANSION AND DETECTION OF CD34 NEGATIVE HSC

Expansion

CD34 positive HSC expansion has been extensively studied[66-72]. $CD34^-$ HSC expansion, however, has not been deeply studied. Stem cell factor and interleukin-11 were used to stimulate normal $CD34^-$ stem cells *in vitro* and showed 1,000-fold expansion of the cells and the acquisition of a $CD34^+$ phenotype by 75% of the cells[7, 17]. Human Lin^- $CD34^-$ cells can be supported for survival and proliferation by cell line, HESS-5, in the presence of fetal calf serum and human cytokines thrombopoietin, Flk-2/Flt-3 ligand, stem cell factor, granulocyte colony-stimulating factor, interleukin-3, interleukin-6, and steel factor. The cells acquire colony-forming ability during seven days of culture, which coincides with their conversion to a $CD34^+$ phenotype[11, 13, 45]. The Stem Cell Factor ligand for tyrosin-kinase receptor c-kit, induces $CD34^-$ HSC to differentiate towards committed hematopoietic progenitor. Differently, IL-6 rather promotes proliferation of $CD34^-$ adherent growing of HSC[30, 73, 74]. Because of the potential of muscle stem cells developing into hematopoietic stem cells, and their relatively easier accessibility, muscle stem cell expansion has been conducted in cultures with epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), FLT-3 ligand, hepatocyte growth factor, and stem cell factor[75].

Counting

Much work has been done in $CD34^+$ cell counting[76-81]. The standardization of $CD34^+$ cell counting has been a difficult issue in research and clinical medicine since its establishment because many factors affect the various methods in different laboratories[78]. In the immunological $CD34^+$ cell counting, the $CD34^-$ HSC has definitely been excluded or missed. The majority of $CD34^-$ stem cells are quiescent fibroblast-like cells, which can be identified in the bone marrow biopsy as "bone lining cells"[11]. Even though the murine models gave a prediction that the majority of stem cells in human cord blood and bone marrow of young children are $CD34^+$, precisely at what age the transition from $CD34^+$ to $CD34^-$ stem cells takes place in man and what is the ratio of $CD34^+$ to $CD34^-$ stem cells in adult human remains to be tested directly on human stem cells. On the other hand, $CD34^+$ cells were previously used to estimate total HSC numbers. However, because of the discovery of $CD34^-$ HSC and the uncertainty of the ratio of $CD34^+$ to $CD34^-$ stem cells in humans, the precision of the estimate and its future role in clinical applications is in doubt. Other methods have been discussed for testing HSC. To investigate the $CD34^-$ HSC detection, one interesting trial was conducted

for circulating human embryonic stem cells in the imunodeficient NOD/SCID animal model[81, 82]. Other issues in $CD34^+$ cell counting include cost and timing, which have been discussed in various publications. In 1995, we conducted HPC/HSC study on the Sysmex SE-9000 automated hematology analyzer[83]. The HPC program was developed on the instrument[84] and evaluated in clinical studies later[85-87]. Our recent study shows that $CD34^+$ and $CD133^+$ cells are located in the same position in the Sysmex IMI channel[88]. Our most recent research (FS Wang, et al, March, 2002) suggests that the HPC/HSC program on the automated hematology analyzer might also cover the $CD34^-$ HSC.

CONCLUSIONS

The discovery of the $CD34^-$ HSC is an important development in hematopoietic stem cell investigation. Even though there are still unclear issues and the need for more research to extensively characterize the human HSC and to clarify the significance of the $CD34^-$ cell population[6, 89-91], current research has provided more profound insights on stem cell biology and related new clinical therapies. Together with the application of other stem cells, it will promote developments in stem cell regenerative medicine. Overall, these insights into the biology of HSC and other stem cells provide novel perspectives for cell and gene therapy of various malignant and non-malignant disorders, and the possibility of replacing defective organ functions with autologous $CD34^-$ stem cells[11] or other stem cells. Because of this, technical developments are transferring to more specialized organizations or manufacturers. The regulations and guidelines in good manufacturing practices (GMP) and good tissue practices (GTP) will help develop better applications and eventually benefit patients[92-94]. Dr. Abkowitz confidently wrote: "This is an exciting time to be a stem cell biologist and a clinician."[95] when commenting on Dr. Körbling's most recent research[34] because we have seen a lot of progress, and are facing many challenges in stem cell research. All of the successes in the field may significantly help human beings in the battle against various diseases.

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