

Self-Renewal of Primitive Hematopoietic Cells: A Focus on Asymmetric Cell Division

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ABSTRACT

The different mammalian blood cell types originate from hematopoietic stem cells (HSCs). HSCs are required in order to sustain the daily production of hundreds of millions of different blood cells fulfilling different functions. Since HSCs have been used for clinical applications for more than three decades, they represent the best studied somatic stem cell entity so far. However, the mechanisms controlling HSC maintenance or differentiation remain largely unknown. Focussing mainly on the process of asymmetric cell division, this chapter will review some of the current knowledge about the mechanisms controlling the decision “self-renewal *versus* differentiation” of primitive human hematopoietic cells.

INTRODUCTION

Somatic stem cells are undifferentiated cells which have been identified in many tissues and organs. They are required for tissue homeostasis i.e., by

creating differentiating daughter cells they provide the fundament for the regenerative capabilities of somatic tissues and organs. To fulfil this function during the entire life span of an organism, somatic stem cells not only have to replenish cells lost by natural turnover or by tissue damaging events, but they also have to maintain the stem cell pool constant i.e., they need to retain their self-renewal capacity. As depicted in Fig. 1, loss of stem cells or of stem cell activities would be as detrimental as their uncontrolled expansion. On the one hand reduced somatic stem cell activities could decrease regenerative capabilities, possibly resulting in tissue degeneration, as for example observed during aging of multicellular

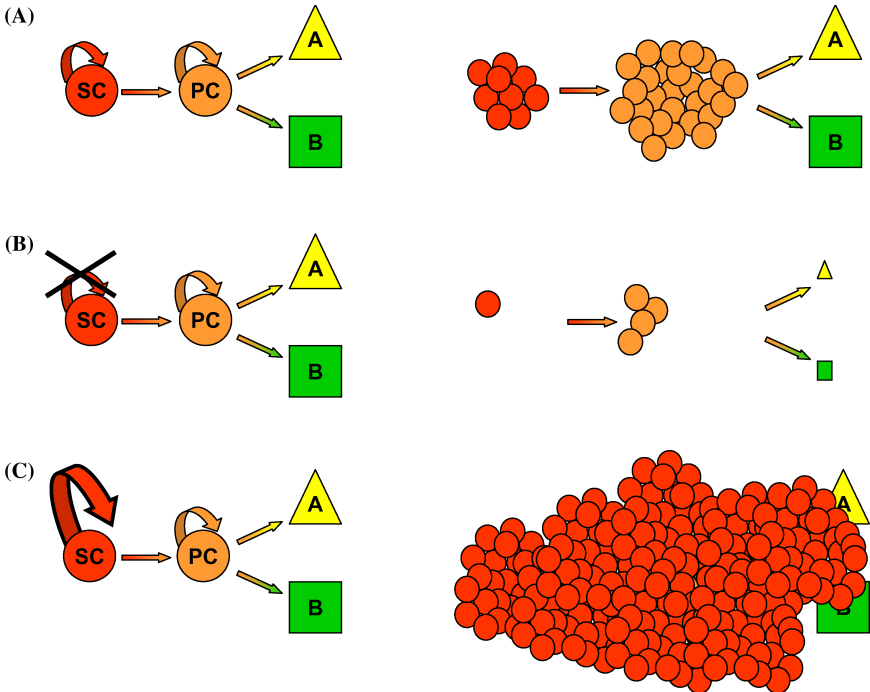


Fig. 1. The decision of somatic stem cells (SC) between self-renewal and differentiation needs to be tightly controlled. **(A)** Adequately controlled self-renewal and differentiation will maintain tissue homeostasis. **(B)** A reduction of the self-renewal capability will decrease the regenerative potential of any given tissue and finally might result in their degeneration. **(C)** In contrast, increased self-renewal capacities might result in tumor formation. PC: Progenitor cell, A: differentiated cell type A, B: differentiated cell type B.

organisms.¹⁻³ On the other hand an uncontrolled expansion could result in tumor formation.⁴⁻⁶ Thus, it becomes evident that the decision between self-renewal and differentiation requires tightly controlled mechanisms. In this respect, it is of fundamental interest in stem cell biology to uncover such underlying mechanisms. Understanding such mechanisms might not only provide new avenues to regenerative medicine, but might also reveal important knowledge that could be helpful to develop new antitumor strategies which could help to increase or decrease the self-renewal capacities of endogenous stem cells or of tumor cells, respectively (Fig. 1).

PRINCIPAL MECHANISMS FOR CONTROLLING THE DECISION “SELF-RENEWAL VERSUS DIFFERENTIATION” OF SOMATIC STEM CELLS

There are different possible strategies for controlling self-renewal *versus* differentiation. For example, following cell division, somatic stem cells could give rise to daughter cells which primarily have identical developmental capabilities. Depending on the activity of extrinsic factors that are provided by the surrounding environment, the initial cell fate could be modulated. A special set of extrinsic factors for example might be required to maintain stem cell activity, whereas other combinations could be responsible to drive the cells into differentiation. By limiting the environmental areas providing stem cell supporting factors, the stem cell pool can, at least theoretically, be kept relatively constant (Fig. 2A). Indeed, research on model organisms such as the fruit fly *Drosophila melanogaster* led to the discovery of stem cell supporting areas in the gonads of these animals.⁷⁻⁹ In analogy to special ecological environments, the ecological niches, these areas are named stem cell niches.

In another scenario, the decision whether a newly formed stem cell daughter retains its stem cell capacity or whether it loses this capacity might depend solely on intrinsic factors. In case a certain concentration of a given intrinsic factor acting as a cell fate determinant is required to maintain the stem cell fate, the distribution of such determinants during mitotic stages could control the presumptive cell fates of both daughter cells. Both daughter cells would inherit different amounts of such cell fate determinants, thus obtaining different developmental capacities, one

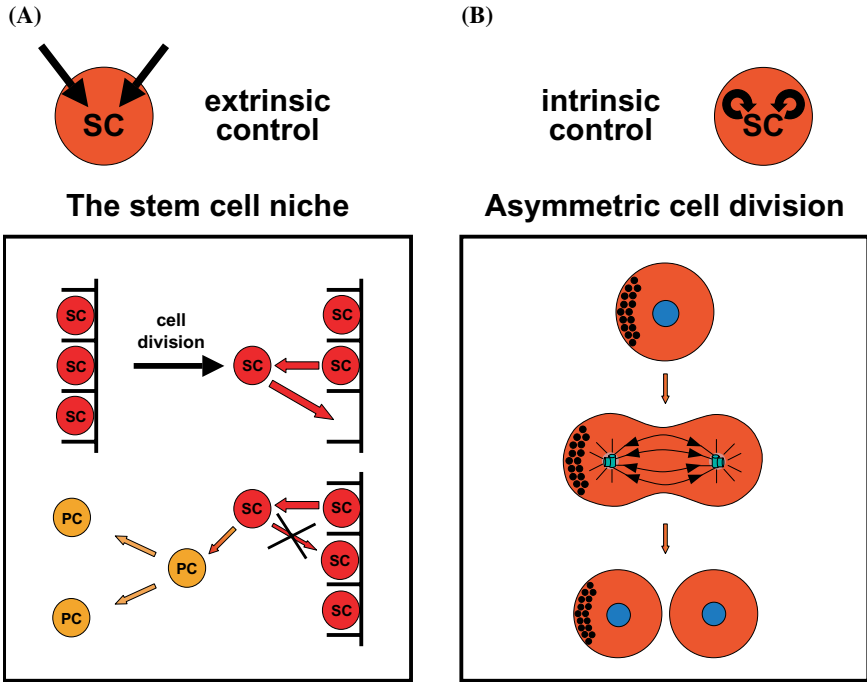


Fig. 2. Extrinsic or intrinsic control of somatic stem cells (SC) to either self-renew or to differentiate. **(A)** The model of the stem cell niche predicts that a particular combination of extrinsic factors being present within the stem cell niches is required to maintain stem cell fate. Stem cells which do not enter an available niche will differentiate. **(B)** Intrinsic cell fate determinants segregate differently into arising daughter cells during asymmetric cell division and control stem cell maintenance versus differentiation.

maintaining the stem cell capacity and one being specified to differentiate (Fig. 2B). Indeed, evidence for such a model has been provided from model organisms, too. In the case of the neural stem cells in *Drosophila melanogaster*, the neuroblasts, it has been shown that during mitotic division proteins acting as cell fate determinants segregate differently into the two daughter cells. As a result of this asymmetric cell division only one of the two daughter cells retains the stem cell capacity whereas the other becomes committed and differentiates.^{10,11}

In the following two paragraphs both models will be discussed in more detail.

Extrinsic Control

The stem cell niche in Drosophila

Studies on germ line stem cells (GSCs) of *Drosophila* contributed largely to the understanding of the concept of stem cell niches. In male flies, six to 12 GSCs reside at the tip of the testis, adjacent to the tightly packed somatic hub cells.^{12,13} In females, two to three GSCs are localized in the tip of each ovariole, the germarium, in close contact to four to seven somatic cap cells.^{14,15} In both sexes, the direct cell-cell contact between the GSC and the hub cells or the cap cells is essential to maintain the GSC fate.^{9,12,13,16} Apart from this cellular contact, female GSCs depend on the secretion of a bone morphogenic protein (BMP) homologue protein Decapentaplegic (DPP) and Glassbottomed Boat (GBB) from the closely attached terminal filament cells.^{8,9,17} Their signaling results in repression of the differentiation factor *bag-of-marbles* (*bam*), whose activity is both necessary and sufficient to drive differentiation of immediate GSC daughters.^{17,18}

Even though a BMP dependence of male GSCs has also been described, the cytokine-like protein Unpaired which is expressed by the hub cells activates the JAK-STAT signaling pathway and, thereby controls GSC self-renewal.^{12,13,19}

In most of their cell divisions GSCs create an anterior located daughter cell maintaining the contact with the cap cells and a posterior located cell losing this contact. While the anterior cell maintains the GSC capacity, the posterior cell normally becomes committed to differentiate. However, if one GSC is lost, both offspring of the remaining GSC can form cellular contacts with the cap cells and thus both are maintained as GSCs.⁹ Experiments in which all GSCs were ablated revealed an alternative strategy for GSC replacement. It was found that depending on the activity of DPP-committed progenitor cells, the cystocytes, can de-differentiate and develop into functional GSCs.^{20,21}

These examples reveal that within a stem cell niche a special combination of extrinsic signals, some of them being provided in a direct cellular contact and others being secreted, act in combination to control the fate of the stem cells and the size of the stem cell pool. In case individual stem cells are lost, they can be replaced either by offspring of remaining stem cells or by progenitor cells that are induced to de-differentiate.

Intrinsic Control

Asymmetric cell division in model organisms

Much of our current knowledge about mechanisms controlling asymmetric cell divisions has come from studies analyzing the development of the zygote of the nematode *Caenorhabditis elegans*. Like in many organisms, the zygote of *C. elegans* displays an asymmetric distribution of determinants that are differentially partitioned to the new blastomers. Here, after fertilization and before the first cleavage, certain vesicles containing germ line specifying determinants, the so-called P-granules, become localized at the posterior pole. Since the zygote divides along the anterior-posterior axis, the P-granules completely segregate into the smaller, posterior blastomere, the so-called P1 cell.²² As a result, the cell inheriting P-granules will form the germline, while the anterior located blastomere, the AB cell, loses this capacity.^{23–25}

The asymmetric segregation of the P-granules depends on a variety of different factors. Initially, a number of such factors have been identified in genetic screens as genes whose activity is required for the proper asymmetric development of the two cell-stage blastomers. As defects in certain genes were found to cause an equal developmental potential of both two cell-stage blastomers, corresponding genes were named *partitioning defect (par)* genes.²⁶ It turned out that different PAR proteins i.e., Par-3 and Par-6, form a complex with an atypical protein kinase C (aPKC) that concentrates in the anterior half of the embryo. By reorganizing the actinomyosin network this complex has been found to be required to establish the anterior-posterior polarity axis in the *C. elegans* zygote.^{27–29} Therefore, it became evident that the establishment of the anterior-posterior axis in the zygote is an essential prerequisite for its asymmetric cell division.

Remarkably, during recent years and as extensively reviewed elsewhere,^{30,31} it turned out that ranging from worm to man the evolutionary conserved PAR/aPKC complex controls establishment and maintenance of cell polarity in a variety of different cell types. In this context, the PAR/aPKC complex also organizes the cell polarity and asymmetric cell division of the *Drosophila* neuroblasts.^{32–36}

Depending on the activity of the PAR/aPKC complex in mitotic stages, the cell fate determinants Prospero and Numb, are localized to the basal

pole of the neuroblast.^{10,11,33} The PAR/aPKC complex also coordinates the binding of Inscuteable (Insc),^{32,33,35,36} a protein which is recruited to the apical membrane in premitotic neuroblasts.³⁷ During mitotic stages Insc controls rotation of the mitotic spindle apparatus in a manner that neuroblast divide perpendicular to the apicobasal cell axis. Depending on this rotation, the apical pole is inherited by one and their basal pole including the cell fate determinants Prospero and Numb by the other arising daughter cell, which then becomes committed to differentiate.^{38,39}

In conclusion, both examples, the *C. elegans* zygote and the *Drosophila* neuroblasts, reveal that asymmetrically dividing cells need to comply with three major prerequisites: firstly, cells need to establish a cell polarity axis, secondly, cell fate determinants need to be localized asymmetrically into the daughter cells, and thirdly, the spindle apparatus needs to be orientated in a manner that the cell fate determinants segregate differently into the arising daughter cells.

STEM CELL NICHEs AND ASYMMETRIC CELL DIVISION ARE NOT MUTUALLY EXCLUSIVE

Although the discussed models are based on either extrinsic or intrinsic cell mechanisms, there are well-investigated cellular systems in which combinations of intrinsic and extrinsic mechanisms orchestrate cell fate decision processes of developing structures. The development of the peripheral nervous system of *Drosophila* provides an excellent model system to study such mechanisms.

In the developing peripheral nervous system, so-called sensory organ precursor cells (SOPs) repetitively divide asymmetrically to form the four different cells of an individual peripheral mechano-sensory organ in a stereotypical manner.⁴⁰

In this context, the cell surface receptor Notch plays a decisive role.^{41,42} Upon binding of its ligands Delta and Serrate the Notch receptor is activated.⁴³⁻⁴⁶ The ligand-binding induces a γ -secretase dependent proteolytic cleavage and a release of the Notch intracellular domain (NICD).^{29,47-52} This domain subsequently enters the nucleus and turns a transcriptional repressor into a transcriptional activator.⁵³⁻⁶⁰

It was found that during SOP development, the Notch-ligands serve as extrinsic signals that are presented to both SOP daughter cells.⁴⁴ Even though both of these cells express Notch and thus are capable to receive the extrinsic signal, only one of them transduces the signal to its nucleus.⁶¹ It turned out that SOPs express the cell fate determinant Numb which, during mitosis, segregates mainly into one of the two daughter cells, where it antagonizes Notch function.^{62–64} Therefore, the intrinsic cell fate determinant Numb antagonizes the extrinsically provided signal that is required to activate the Notch signaling pathway.

THE HEMATOPOIETIC STEM CELL COMPARTMENT

Like any other somatic cell type with stem cell properties, HSCs are able to self-renew and differentiate into specialized cell types. In contrast to other somatic stem cells, HSCs are successfully used in clinical trials for more than 30 years.⁶⁵ Initially, a potential clinical usage of human HSCs was indicated when lethally irradiated mice were rescued by the transplantation of cell fractions containing HSCs; the transplanted HSCs led to a complete reconstitution of the murine blood system.^{66,67} Since then basic research and clinical trials improved our knowledge about HSC biology as well as their clinical applications, so that nowadays HSC containing cell fractions of different sources are routinely used to transplant different cohorts of patients.⁶⁵

The Hematopoietic Stem Cell Niche

Triggered by their enormous clinical relevance, many attempts have been performed to formulate conditions allowing *ex vivo* HSC expansion. As extensively reviewed elsewhere,⁶⁸ almost all of these attempts failed to expand HSCs in larger amounts. However, differentiation of primitive hematopoietic cells is slowed down upon culture on certain stromal cells and the primitive potential of such HSC-enriched cell fractions can be maintained for at least several weeks in culture.^{69–72} In contrast, it seems that HSCs can expand *in vivo*. As already mentioned,

myeloablated patients can be cured by HSC-containing grafts for their normal lasting life time.⁶⁵ Moreover, small numbers of HSCs can be transplanted sequentially in animal models without losing their stem cell potential, leading to successful regeneration in each generation.⁷³ These observations demonstrate that the environment is important in controlling the development of HSCs and their progeny. In this context, Raymond Schofield proposed as early as in 1978 the stem cell niche hypothesis, in which the stem cell is seen in association with other cells which determine its and its progeny behavior. As long as stem cells can occupy stem cell niches they can be maintained as stem cells, otherwise they get committed to differentiate.⁷⁴

Indeed, evidence for HSC-niches has recently been reported, with osteoblasts forming putative bone marrow (BM) niches in the endosteum, and sinusoidal endothelial cells the vascular HSC-niches in the spleen and BM.⁷⁵⁻⁷⁷ It seems that the receptor tyrosine kinase Tie2 which is expressed on HSCs and its cognate ligand Angiopoietin-1 produced by the osteoblasts play a crucial role in these niches.⁷⁸ Furthermore, osteoblasts express the Notch ligand Jagged-1 which can activate the Notch signaling pathway. This, together with the fact that in cocultures the long-term supportive effect of osteoblasts is strongly reduced by blocking Notch activation, suggests that another important function of HSC niches is the activation of the Notch signaling pathway in the stem cell.⁷⁵ More recent data that are reviewed elsewhere suggest additional roles of the Wnt signaling pathway, and of HSC-supporting factors such as the angiopoietin-like (Angptl) proteins which might be involved in maintaining the stem cell pool.^{79,80}

ASYMMETRIC CELL DIVISION OF PRIMITIVE HEMATOPOIETIC CELLS

Before the concept of the HSC niche was improved, it was widely assumed that HSCs divide asymmetrically to give rise to one daughter cell maintaining the stem cell capability and to another cell being committed to differentiate. Observations supporting this hypothesis are presented in the next paragraph.

Primitive Human Hematopoietic Cells Can Adopt Different Cell Fates

First evidence that HSCs might divide asymmetrically came from studies of the group of Ogawa.⁸¹⁻⁸³ They separated daughter cells of primitive hematopoietic cells and compared their proliferation rate and differentiation capacity. It was observed that in some cases sister cells developed colonies of different types and/or of different sizes. They assumed that these differences might be the result of stochastic processes that decide whether stem cells self-renew or differentiate.⁸¹⁻⁸³ Differences in the development of separated daughter cells were also found by the group of Landsdorp.⁸⁴ They separated progenies of human umbilical cord blood derived $CD34^+/CD45RA^{low}/CD71^{low}$ cells, a cell fraction highly enriched for primitive hematopoietic cells, and cultured them under identical or different culture conditions. Similar to the group of Ogawa, they found that a small fraction of initially deposited cells gave rise to siblings that formed different colony types. In their subsequent studies the development of individual fetal liver-derived $CD34^+/CD38^-/CD71^{low}/CD45RA^{low}$ cells were analyzed. In this context Brümendorf and colleagues⁸⁴ recognized that in some cases progenies of deposited cells followed different proliferation kinetics i.e., some of the cells showed fast and others slow proliferation kinetics. Since slow dividing cells showed higher expansion rates than fast dividing cells, they were judged to be the more primitive daughter cells. Next, the group subcloned $CD34^+/CD38^-$ cells of slow dividing cell clones and observed that arising daughter cells again could be discriminated in terms of their proliferation kinetics. The authors assumed that differences in the cell fate of arising daughter cells might continuously and intrinsically be generated by asymmetric cell divisions, in which similarly to asymmetric cell divisions in *Drosophila*, molecules acting as cell fate determinants are differently distributed during mitosis. However, they also discussed that there are alternative explanations for their observations, such as postmitotic mechanisms.⁸⁴

In another set of experiments the group of Ho²⁹ labelled $CD34^+/CD38^-$ cells derived from human fetal liver, umbilical cord blood and adult bone marrow with the membrane dye PKH-26. Studying the proliferation

kinetics of these cells over time, they observed that depending on the cytokines used, deposited cells performed their first *in vitro* cell division after 36 to 38 hours (in the presence of late acting cytokines) or after 48 to 50 hours (in the presence of early acting cytokines) post-seeding. However, in a cytokine independent, but cell source dependent manner, approximately 20%–40% of the CD34⁺/CD38⁻ cells gave rise to daughter cells with asynchronous proliferation kinetics.²⁹ In this context, functional studies on umbilical cord blood-derived CD34⁺/CD38⁻ cells revealed that a higher content of more primitive hematopoietic cells gives rise to daughter cells with asynchronous proliferation kinetics than more mature progenitor cells. Confirming the data of Brümmendorf and colleagues (1998), slow dividing cells were found to be more primitive than fast dividing cells.⁸⁵

In collaboration with Ho's group, our group then performed cell separation studies.⁸⁶ Since umbilical cord blood-derived primitive hematopoietic cells contain higher regenerative abilities than corresponding adult cells derived from BM or from the peripheral blood of G-CSF mobilized stem cell donors,^{87,88} mainly umbilical cord blood-derived cells were used for these studies. To specifically analyze more primitive hematopoietic cells it was first tested whether, apart from cell surface antigens, cell culture conditions might also help to enrich for more primitive hematopoietic cells. Indeed, it was shown that in the presence of late acting cytokines mainly more mature CD34⁺ cells are enforced to perform their first *in vitro* cell divisions within the first five days of culture, whereas most of the more primitive CD34⁺ cells remain quiescent for at least the first five days. In this context it should be mentioned that within the presence of early acting cytokines, almost all CD34⁺ cells are induced to perform their first *in vitro* cell division between a 48- to 72-hour culture.⁸⁶

For cell separation studies, individual CB-derived CD34⁺/CD38⁻ cells were sorted into individual wells of 96-well plates and were cultured in the presence of late acting cytokines. Within 24 hours, siblings of cells which performed their first cell division between culture day 5 and day 10 were separated from each other and individually expanded on stromal cells (AFT024), which support maintenance of primitive hematopoietic cell fates.⁷¹ Next, the offspring of each of the separated

cells were tested for their capacity to form myeloid as well as lymphoid cell types. In case initially deposited or separated daughter cells gave rise to myeloid as well as to lymphoid offspring they were retrospectively termed as myeloid-lymphoid initiating cells or briefly as ML-IC.⁷¹ More than 80% of the initially deposited cells which were retrospectively judged as ML-ICs gave rise to daughter cells, one of which also revealed the ML-IC potential, and one of which showed a reduced developmental capacity. Therefore, these data suggest that at the point of time the ML-IC siblings were separated, they were differently specified, one still retaining the ML-IC capacity, whereas the other was more committed.⁸⁶

Even though these data are compatible with the model of asymmetric cell division as it was initially discussed by Brümmendorf and colleagues, the observed differences might also result from post-mitotically acting processes i.e., immediately after mitosis arising daughter cells might contain identical developmental capacities that might be altered during the time between the initially deposited cell had finished its mitosis and before the daughter cells got separated.^{84,86} Since both possibilities, asymmetric cell division and post-mitotic mechanisms, could explain the outcome of the delineated studies, it remained an open question whether primitive hematopoietic cells indeed could divide asymmetrically.⁸⁶

Cell Polarity of Primitive Hematopoietic Cells

As discussed before, an important prerequisite for asymmetrically dividing cells in model organisms is that they are polarized. In the case that observed differences in the development of primitive hematopoietic cell siblings are indeed established by asymmetric cell divisions, they should obey similar principles than other asymmetrically dividing cells and thus should contain a cell polarity axis. Indeed, while freshly isolated CD34⁺ cells regularly appear as small round cells without any apparent indication of cellular polarity, cultured CD34⁺ cells adopt an elongated morphology, forming a leading edge on one end and a structure named uropod at the other end.⁸⁹ This morphology resembles the migrational phenotype of immunoreactive leukocytes. Moreover, certain drugs such as the

PI3K-inhibitor Ly294002 that interfere with processes required to establish polarized cell shapes of cultured CD34⁺ cells, also interfere with their *in vitro* migratory capabilities.⁸⁹

Since several proteins were known to adopt a polarized distribution in migrating leukocytes and several of these proteins are expressed on primitive hematopoietic cells, their distribution was studied on cultured CD34⁺ cells. In this context it could be shown that the stem cell marker CD133/Prominin-1 colocalizes with known uropod markers, the cell adhesion molecules CD43, CD44, CD50 and CD54, at the tip of the uropod. In contrast, the chemokine receptor CXCR4 and the ganglioside GM3 are highly enriched at the opposite pole, the leading edge, whereas the cell surface proteins, CD34 and CD45 show an even distribution over the entire cell surface of the CD34⁺ cells.⁸⁹

Next, using some of these antigens, the cell polarity axis in mitotic cells was studied. CD43, CD44, CD50 and CD54 together with CD133 become highly enriched in the cleavage furrow and at later mitotic stages on the cytoplasmic bridge connecting the arising daughter cells, on the so-called midbody.⁹⁰ This set of data demonstrates the existence of a cell polarity axis in dividing CD34⁺ cells.

However, since in dividing CD34⁺ cells none of the investigated antigens revealed an asymmetric distribution, it still remained an open question whether primitive hematopoietic cells can divide asymmetrically. Either the cells do not divide asymmetrically or the investigated cell polarity markers are not inherited differently in asymmetrically dividing CD34⁺ cells.

Identification of Asymmetrically Segregating Antigens

Studying the proliferation kinetics of the cultured CD34⁺ cells, it was realized that initially most of the CD34⁺ cells also express CD133. However, with the onset of cell divisions between culture day 2 and day 3, a novel cell population appears whose cells have largely lost their cell surface expression of CD133, but not that of CD34 (Fig. 3). Remarkably, the proportion of this population increases over time and the underlying kinetics are compatible to a model in which — according to the functional studies described above — approximately 70% of the more primitive cells divide

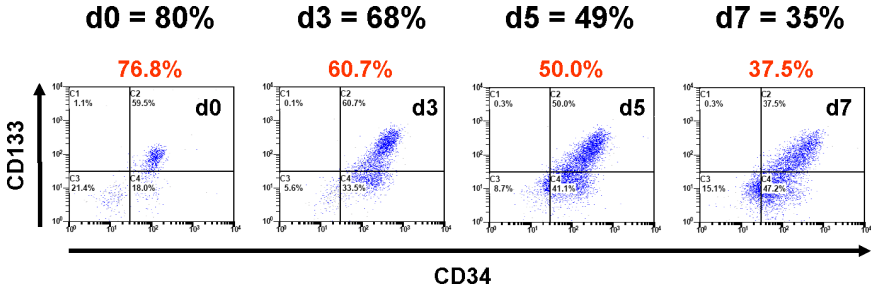


Fig. 3. Hypothetical and measured content of primitive hematopoietic cells within the fraction of cultured CD34⁺ cells. As pointed out in the text functional analyses revealed that approximately 30% of primitive hematopoietic cells give rise to daughter cells following different proliferation kinetics and/or realizing different cell fates. Assuming that (i) 30% of the primitive hematopoietic cells (CD34⁺/CD133⁺) divide asymmetrically, giving rise to a primitive (CD34⁺/CD133⁺) and a more specified daughter cell (CD34⁺/CD133^{low/-}), (ii) starting at culture day 2 all CD34⁺ cells divide once a day, and (iii) approximately 80% of the freshly isolated CD34⁺ cells express CD133, the content of CD34⁺/CD133⁺ cells at a given culture day n can be calculated with the formula: **content of CD34⁺/CD133⁺ cells = $0.8 \times (0.7 + 0.15)^{n-2}$** . The theoretical content of CD34⁺/CD133⁺ cells within a CD34⁺ cell population at a given culture day is given in black letters, that of the experimentally measured content in red letters. Corresponding plots are shown below.

symmetrically to create two CD34⁺/CD133⁺ cells and approximately 30% asymmetrically to create a more primitive CD34⁺/CD133⁺ daughter cell and a more mature CD34⁺/CD133^{low/neg} cell.^{86,90} Since in none of the dividing cells studied CD133 revealed an asymmetric cell surface distribution, CD133 itself was not a candidate for an asymmetrical segregating protein. However, due to the intriguing population kinetics it was hypothesized that any other cell surface molecule being differently expressed on CD34⁺/CD133⁺ and CD34⁺/CD133^{low/neg} cells, in principle provides a candidate for an asymmetrically segregating molecule.

In this context, a flow cytometry based screen identified several cell surface antigens such as CD53, CD62L, CD63 and CD71 being differently expressed on CD133⁺/CD34⁺ and CD133^{low/-}/CD34⁺ cells. On dividing CD34⁺ cells the tetraspanins CD53 and CD63 as well as the transferrin receptor (CD71) and the L-selectin (CD62L) showed an asymmetric distribution on 20%–30% of the mitotic CD34⁺ cells studied,⁹⁰ strongly suggesting that human primitive hematopoietic cells indeed can divide

asymmetrically. Even though it is not known yet, whether the identified molecules are causally linked to the process of asymmetric cell division, three of them (CD53, CD63 and CD71) provide physical links to the endosomal compartment.⁹⁰ This coupled with the more recent finding that a subset of endosomes, the ones expressing the early endosomal antigen-1 (EEA-1), segregate mainly into the anterior part of the asymmetrically dividing zygote of *C. elegans*,⁹¹ suggests that asymmetric segregation of endosomes might provide a more general mechanism in asymmetrically dividing cells.⁹⁰ Supporting this notion, it just recently has been published that an asymmetric segregation of certain endosomes can also be observed in asymmetrically dividing sensory organ precursor cells of *Drosophila melanogaster*.⁹²

Asymmetric Cell Division and CD133

According to previous findings, endosomes can segregate asymmetrically in dividing cells. Since endosomes are required for molecular trafficking within the cells, it appears likely that several proteins, including proteins with extracellular epitopes, are differentially distributed during the course of asymmetric cell divisions. In this context, the asymmetrical distribution of certain proteins might occur on the intracellular level rather than on the cell surface. Since asymmetrically segregating proteins were identified by comparing the cell surface expression of different proteins on CD34⁺/CD133⁺ and CD34⁺/CD133^{low/neg} cells,⁹⁰ it is an interesting question how the differences in the CD133 content itself are established. In principle, this might be mediated by post-mitotic mechanisms in which only the more primitive CD34⁺ cells can re-express and/or maintain CD133 expression. Alternatively, differences might also be established by the asymmetric segregation of the CD133 protein itself. Even though we did not find any indication for an asymmetric segregation of the CD133 on the cell surface, it cannot be excluded that an intracellular fraction of CD133 segregates differently into the daughter cells to establish the observed differences. Indeed, Fonseca *et al.* (2008) analyzed the intracellular CD133 distribution in dividing primitive hematopoietic cells and observed a colocalization of CD133 and CD63 within the intracellular compartment. Confirming previous data, they found that in approximately 20% of the

mitotic cells studied, both CD63 and CD133 mainly segregate into one of the arising daughter cells.^{90,93} Therefore, in asymmetrically dividing primitive hematopoietic cells CD133 can indeed segregate asymmetrically.

Endosomes are Involved in Cell Fate Specification Processes

Since asymmetrically segregating proteins were identified by comparing the CD34⁺/CD133⁺ and CD34⁺/CD133^{low/neg} cell fractions,⁹⁰ the question was whether the asymmetric segregation of the identified proteins and most likely that of certain endosomes might correlate with the cell fate of corresponding cells. To get some insight, the potential of the cells was compared in long-term culture initiating cell (LTC-IC) assays, an *in vitro* read out system to estimate the content of primitive myeloid cells. Almost all cells with LTC-IC capacities were enriched within the CD34⁺/CD133⁺ cell fraction.⁹⁰ According to these results, the asymmetric distribution of the proteins identified seems to correlate with the cell fate the cells finally adopt.

Evidence that endosomes and endosomal proteins are involved in cell fate specification processes has been obtained from model organisms. Within the developing wing of *Drosophila melanogaster* an anterior-posterior gradient of the TGF- β homologue protein DPP controls the fate of the wing epithelial cells in a concentration-dependent manner. Upon binding to its receptor Thickveins (Tkv), a type I TGF- β receptor, DPP induces the phosphorylation of the R-Smad transcription factor Mad and recruits a common Smad to become an active transcription factor that then translocates into the nucleus and induces the expression of target genes. The endosomal protein Smad-anchor-for-receptor-activation (SARA) is required for the recruitment of R-Smads to type I TGF- β receptors.⁹⁴ In mammals as well as in *Drosophila*, Sara accumulates in early endosomes; which in the *Drosophila* wing epithelial cells also contain DPP and its receptor Tkv.^{95,96} During mitosis of normally developing wing epithelial cells, these endosomes equally segregate into the arising daughter cells.⁹⁵ However, even though in Sara mutant wing epithelial cells DPP and Tkv containing endosomes still exist, their distribution into arising daughter cells is randomized. Frequently, daughter cells obtain different amounts of Tkv containing endosomes, and in correlation with that they adopt different

cell fates. Cells with a higher content of Tkv containing endosomes adopt more anterior and those with a lower content more posterior cell fates.⁹⁵

Homeostasis Requires Orchestration of Extrinsic and Intrinsic Cues

In addition to asymmetric division of primitive hematopoietic cells, data suggests that extrinsic signals may play a role as well. The observation that a higher percentage of primitive hematopoietic cells cultured on the stromal cell line AFT024 create daughter cells with different proliferation kinetics than corresponding cells that were cultured under stroma-free conditions, suggests that extrinsic signals can modulate intrinsic cell fate specification processes.⁹⁷ Hence, special combinations of extrinsic and intrinsic signals seem to regulate cell fate specification processes within the primitive hematopoietic compartment. Thus, it becomes an important question of how extrinsic and intrinsic cues cooperate to regulate mammalian hematopoietic homeostasis. It remains a major challenge to define culture conditions allowing the expansion of HSCs. Therefore a better understanding of the processes and mechanisms controlling asymmetric cell divisions within the primitive hematopoietic system may help us to improve and control cell culture conditions allowing optimized expansion of HSCs *ex vivo*.

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