Intracellular Factors Regulating Activity of Hemopoietic Stem Cells

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The problem of polypotent hemopoietic stem cells (HSC) is crucially important for biology and medicine and this problem will hardly be solved in the nearest future.

HSC always faces the "what-to-do" question: to maintain itself, to differentiate, to go into apoptosis, or to migrate. These four possible ultimate pathways for each stem cell are associated with cascade changes in its functional activity, including modulation of its membrane phenotype, activity of transcription factors, responsiveness to growth factors, proliferative activity, prolipherative potential, and linear restriction (readiness to differentiation in this or that direction).

Under some experimental conditions HSC are insensitive to regulatory factors, which led to a conclusion that proliferation and differentiation of HSC are stochastic processes not depending on inducing factors of the microenvironment. Taking this into consideration, we can assume the existence of intrinsic regulatory mechanisms in HSC operating without appreciable involvement of exogenous factors.

Studies aimed at evaluation of the role of genes whose expressed in HSC only will help to solve the problem of HSC. It is more likely, the products of these genes act via an autocrine mechanism. Isolation and purification of these products will allow more specific modulation of the proliferation and differentiation potential of HSC populations. The most plausible candidate gene for this role is hiwi gene expressed in $CD34^+$ HSC cells. It was found that under conditions promoting HSC differentiation and exit from the compartment of stem cells these cells lost the capacity to express hiwi gene. This expression was not determined in stromal and mesenchymal stem cells of the bone marrow, which attests to its specificity for HSC and characterizes it as an intracellular regulator of HSC self-maintenance [62]. The function of this gene probably consists in the selective maintenance of HSC population in the native state. Another gene encoding transcription factor STAT5 and different isoforms of its products are responsible for differentiation of HSC into committed precursors. In mice lacking STAT5A and STAT5B the capacity to splenic colony formation was impaired and populations of committed precursors were reduced against the background of high proliferative activity of HSC [64]. It was hypothesized that intracellular adaptor Lnk protein also acts as an autocrine antiproliferative factor. Upon binding with c-Kit this protein is phosphorylated and selectively inhibits c-Kit-mediated proliferation via attenuation of Gab2 phosphorylation and activation of cascade reactions of mitogen-activated protein kinase. Taking into account the regulatory role of this protein in B cell production, it is probably involved into differentiation of HSC towards B cell lymphopoiesis [66]. Another adaptor protein Grb2 interacting with SH2-containing inositol-5'-phosphatase can also play a role in modulation of activation threshold of HSC. An isoform of this enzyme expressed in primitive HSC, but not in committed precursors was identified [71].

The Wnt gene family consisting of 19 members [63] also participates in processes determining HSC development. Most likely, products of these genes are involved into regulation of hemopoiesis, because their expression was found in embryonic hemopoietic organs (yolk sac, embryonic liver and bone marrow). Wnt-5α and Wnt-10β mRNA was detected in primitive hemopoietic precursors from embryonic liver $AA4^+$ and only Wnt-10β in $AA4^+Sca^+Kit^+$ cells from embryonic liver capable of long-term maintenance of the hemopoiesis in the recipient [2]. Primitive precursor

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cells $CD34^+Lin^-$ from human embryonic and adult bone marrow express only Wnt-5α mRNA [72]. It should be noted that in all studied cell populations enhanced expression of these genes responsible for production of regulatory glycoproteins led to activation of their proliferation. These data and the presence of specific receptors encoded by Frizzled gene family on stem cells [5] suggest that regulatory effects of products of this gene family can be mediated via not only paracrine, but also autocrine mechanisms. Most likely, Wnt genes participate not only in the regulation of cell proliferation. Experiments on QCE6 cells (aviam mesodermal stem cells), precursors for all blood cells under certain conditions of culturing, showed that suppression of Wnt-11 gene expression with a specific antisence molecule determines primarily macrophageal differentiation of precursors and the absence of erythrocytes in this culture. Addition of the product of this gene induced predominant differentiation of cells into erythrocytes and monocytes [7].

An important role in processes of HSC proliferation and differentiation is played by homeobox gene LH2 (Lhx2). Products of this gene are presumably responsible for HSC self-maintenance. It was demonstrated that HSC derived from embryonic stem cells with transduced LH2 gene are characterized by considerably longer self-maintenance compared to control HSC. LH2-transduced HSC did not lose differentiation potential under the effect of cytokines and are insensitive to the inhibiting effect of TGF-β_1. It was hypothesized that long-term self-maintenance of HSC is determined by an unknown factor, a product of LH2 gene [54,55]. Markers of HSC characterizing different stages of their differentiation with consideration of their self-maintenance capacity were described.

It is quite possible that expression of receptors to regulatory molecules on HSC is also controlled by intracellular factors. It was found that tyrosine kinase receptor Flk-2 is absent on most primitive HSC from murine bone marrow characterized by long-term self-maintenance, but was detected on more differentiated HSC subpopulation capable of only short-term self-maintenance [12]. Different stages of HSC differentiation are characterized by expression of cytokine receptor genes (a total of 17 genes were studied) [6].

Apart from factors enhancing self-maintenance capacity of HSC, these cells produce factors with opposite activity. The latter include representatives of two families of cyclin-dependent kinase inhibitors: Cip/Kip (inhibitors p21 and p27) and INK4a (inhibitors p16INK4a and p19ARF). p16 and p21 inhibit phosphorylation of retinoblastoma protein and arrest cell division in G_1 phase (restriction point) [29]. Mouse HSC lacking this protein demonstrated more rapid colony growth and increased capacity to the formation of secondary colonies [39]. The effect of HTm4, a new cell cycle regulator in HSC, a representative of the HTm4/CD20/FcξRIβ subfamily, is also associated with phosphorylation of cyclin-dependent kinase 2 and cell arrest in the G_0/G_1 phase of the cell cycle [18].

An important role in proliferation and differentiation processes in HSC and committed precursors is played by the family of GATA transcription factors. The effect of these factors is based on their ability to bind consensus DNA sequence (T/A)GATA(A/G) via a highly conservative DNA-binding domain consisting of amino- and carboxy-terminal zink-fingers [70]. It was shown that GATA-2 factor promotes expansion of HSC in a culture of mouse embryonic stem cells [34]. Enhanced expression of this factor determined predominant differentiation of HSC towards erythro- and thrombopoiesis against the background of blocked macrophageal differentiation. Increased expression of GATA-2 in HSC is associated with their decreased proliferation and differentiation capacities, which attests to possible involvement of this factor in the formation of resting state in HSC. Increased expression of GATA-2 in immature erythroid cells blocks their further differentiation [51]. It is quite possible that proliferation and differentiation of HSC and differentiation of erythroid elements are associated with a decrease in GATA-2 gene expression below a certain critical level. Similar effect on differentiation processes is produced by GATA-1, but unlike GATA-2, this factor has no effect on expansion of HSC. Expression of GATA-3 was mainly attributed to lymphoid precursors and T cells [27]. Transduction of GATA-3 gene into mouse polypotent HSC determined their predominant differentiation towards erythro- and thrombopoiesis against the background of blockade of myeloid and lymphoid differentiation pathways. Proliferative activity of HSC was completely blocked. In this case, GATA-3 gene product acted similarly to GATA-1 gene product. It can be assumed that increased expression of both these genes can determine exhaustion of the HSC pool [11].

A unique role in proliferation and differentiation of HSC is played by transcription factors belonging to the Hox family. These factors are expressed only in primitive HSC, *e.g.* $CD34^+CD38^-$ bone marrow cells. It was demonstrated that the absence of functionally active Hoxa9 gene is responsible for disturbance in HSC differentiation towards lymphoid, myeloid, and erythroid cells [37]. Enhanced expression of Hoxb3 gene in HSC suppresses lymphoid, but stimulates myeloid differentiation, which is accompanied by progressive myeloproliferation [60]. Enhanced expression of Hoxa10 gene is associated with disturbed differentiation of megakaryocytes, macrophages, and B cells and with myeloproliferative syndrome [68]. It was found that enhanced expression of Hoxb4 gene in HSC

determines their increased proliferative, repopulation, and regeneration activities, without changes in differentiation capacities and sensitivity to regulatory mechanisms. This is probably related to shortening of the cell cycle in HSC [1]. Expression of Hox genes is absent in embryonic stem cells, but appears during their differentiation towards primitive HSC. Most these genes (a total of 39 genes in 4 clusters: A, B, C, and D) are not expressed in more mature committed precursors. It was hypothesized that proteins encoded by Hox genes do not directly bind to DNA, but act via the formation of heterodimers and trimers with cofactors encoded by Pbx and Meis. Expression of these genes appears in HSC practically simultaneously with expression of Hox genes [53].

Notch gene family also belongs to genes responsible for the choice between differentiation and self-maintenance for cell elements, specifically stem cells [42]. Products of these genes are a group of highly-conservative proteins acting as surface cell receptors and direct regulators of gene transcription. Activation of these receptors with specific Jagged ligands via a series of intermediate processes activates genes acting as negative regulators of expression of differentiation-specific genes. *In vitro* experiments showed that the ligand-receptor interaction in the absence of growth factors reduced colony-forming capacity of $CD34^+$ HSC, incorporation of ^3H-thymidine into these cells, and their enter into S phase of the cell cycle. In the presence of growth factors ($IL\text{-}3^+$, $IL\text{-}6^+$, SCF^+, G-CSF), Jagged1 had no effect on activity of $CD34^+$ HSC, their expansion, and formation of hemopoietic colonies. In the presence of G-CSF ligand, Jagged1 stimulated the formation of erythroid colonies [73]. In the absence of growth factors, the maintenance of HSC in the immature state is probably regulated via Notch1 receptors. This assumption was confirmed in experiments with transfer of human HSC treated with Jagged1 ligand to immunodeficient mice. These manipulations improved HSC survival and their expansion in the recipient organism [32]. It can be hypothesized that either Notch/Jagged interactions in the presence of growth factor cannot overcame their stimulating effects, or growth factors suppress expression of Notch receptors on HSC. Intracellular interactions of transcription factors induced via growth factor receptors and Jagged1 resulting into inhibition of signaling from Notch receptors. Some examples of antagonistic interactions of two genes are known. It was found that *eed* gene product inhibits, while Bmi1 gene product stimulates proliferation of mouse HSC. It was assumed that proliferation of HSC is regulated by stoichiometric relationships between A-mediated stimulatory complex and B-mediated inhibitory complex of cell proliferation. Products of these genes belonging to the same group Polycomb-Group, are components of different complexes [38].

Published data suggest that function of intracellular regulatory molecules does not depend on exogenous factors. In light of this, the studies of the main regularities in the function of these factors are very important. Without solving this problem, differentiation processes in HSC seem stochastic. D2 mice are characterized by greater number of HSC participating in colony formation (cobblestone area-forming cells), higher percentage of S-phase HSC, and their more intensive migration into the blood after injection of G-CSF, compared to Bl6 mice. Moreover, HSC from D2 mice exhibited higher repopulation capacity in lethally-irradiated recipients, compared to HSC from Bl6 mice. After 5-fluorouracil treatment hemopoiesis more rapidly recovered in D2 mice. These functional differences in HSC activity were retained in F_1-hybrid recipients receiving a mixture of embryonic HSC from both parent genotypes. The authors concluded that this autonomy can be determined by some genes expressed in HSC and regulating their behavior. It is most likely, that migration capacity of HSC is also determined by genetic factors. Considerable quantitative differences between different mouse genotypes by the number of HSC mobilized into peripheral blood from the bone marrow in response to G-CSF were reported. The results obtained on F_2-hybrids suggest that the phenotype of HSC mobilization into the blood is a complex sign with numerous genetic loci [13,59] (a group of genes regulating stem cells ratio). It was found that in chimeric mice obtained by aggregation of morulae from D2 and Bl6 mice, HSC of Bl6 genotype demonstrated autonomic behavior in long-term cultures [44]. It is still unknown which genes are involved in these intracellular regulatory mechanisms. In this case, genes stimulating repopulation activity of HSC (Hoxb4, Bcl-2) and genes inhibiting this activity can play a role. It was demonstrated that expression of protooncogen c-fos and deficit of cyclin-dependent kinase p21 inhibitor reduce proliferation activity of HSC. The effect of genotype manifested in ontogenetic behavior of HSC population. It was found that in DBA/2 mice the number of HSC and the percentage of S-phase HSC in embryos and adult animals (<1 year) surpassed the corresponding values in C57Bl/6 mice. At the same time, during aging (to 2 years) the number of HSC in the bone marrow still increased in C57Bl/6 mice, but considerable decreased in DBA/2 mice. These age-related peculiarities were observed in BXD recombinant inbred mice, offspring of the above mouse strains [14]. The same authors revealed a relationship between the life span of different mouse strains and population characteristics of HSC, although no inter-strain differences in parameters of the peripheral blood

were found. The animals with short life span were characterized by similar content of more mature HSC and their high proliferative activity and had either low or high content of more primitive HSC. The mice with long life span principally differed from the above two groups by considerably reduced proliferative activity of more mature HSC and medium content of primitive HSC. The authors adduce proofs on genetic control over the size of HSC pool with mapping of the responsible loci in chromosome 18 [15]. Later, the same authors published findings suggesting age-dependent effects of the genotype on the size of HSC population: in young animals the size of HSC pool is regulated by loci in chromosomes 1, 3, 5, and X, while in old animals this parameter is related to chromosomes 2, 7, 14, 15, and 18 [23].

When the fate of the offspring of a single HSC was studied, the following results were obtained. Primary clones of $CD34^+CD38^-$ HSC from embryonic liver disintegrate into slow and rapidly growing clones. Slow growing clones (16% clones) were characterized by higher content of HSC. Similar data were obtained when studying the fate of single HSC from a slow growing primary clone. The authors believe that the subpopulations of HSC from rapidly growing clones are more committed to differentiation than cells from slow growing clones. Asymmetrical division of each cell consisting in uneven distribution of cell organelles, transcription factors, and other regulatory molecules, including Notch-1, Jagged1, *etc*., was hypothesized. Intracellular regulatory processes can play a role in determination of the fate of cell offspring, *i.e.* culturing conditions were similar in all cases [8]. Published data suggest that 3-20% cell divisions at the level of HSC are asymmetrical. At the same time, excessive cell proliferation and expansion of more committed hemopoietic precursors are probably characterized by symmetrical cell division [41,43,65]. Other authors cultured *in vitro* solitary $CD34^+CD38^-$ HSC from embryonic liver in the presence of different combinations of regulatory molecules. The rate of mitotic cycle, efficiency of colony-formation, and proportion of asymmetrical divisions were changed depending on cytokine "cocktail", on ontogenetic age of HSC (percentage of asymmetric divisions was higher for HSC from embryonic liver, compared to those from umbilical vein or adult bone marrow), while the index of asymmetric division (the ratio of asymmetric divisions to the total number of dividing cells) was constant in most cases. Authors concluded that the decision about differentiation depends on extracellular factors. Symmetrical pattern of early divisions, the fate of daughter cells, *i.e.* decision of self-renewal, are most likely controlled by intracellular factors [28]. Analysis of several thousands genes revealed differences in the expression of many genes between HSC characterized by self-renewal capacity and early multipotent precursors losing this ability [47]. Disturbed expression of some genes is probably responsible for impaired capacity of polypotent HSC in NZB mice to generate committed lymphoid offspring and less efficient migration of cells from the bone marrow into the thymus. This latter phenomenon is probably a mechanism triggering the formation of autoimmune pathology in animals of this strain [24].

Being a target for many cytokines, HSC can produce regulatory molecules. These molecules via auto-, intra-, retro-, and paracrine regulatory mechanisms determine autonomy of HSC behavior. This autonomy is a mechanism maintaining the pool of HSC and protecting this pool from exhaustion under the effect of different external and internal factors.

There are interesting data on the expression of c-Kit-receptor ligand gene (stem cell factor-SCF, steel factor) in $CD34^+$ HSC. These data attest to the possibility of maintenance of proliferative activity of HSC at the expense of autocrine production of a factor stimulating this activity. It was found that blockade of c-Kit ligand gene expression with oligodeoxynucleotide inhibited the formation erythroid (BFU-E) and mixed colonies (CFU-Mix) by$CD34^+$ c-Kit^+-cells from the bone marrow, but did not affect the growth of granuloid colonies (CFU-GM). Inhibition of stk1 gene encoding tyrosine-kinase ligand alone in stem cells had no effect on colony-formation process. However, blockade of these two genes with antisences led to additive inhibition of the growth of granuloid and mixed colonies, but had no effect on the growth of BFU-E [57]. Authors supposed the existence of an autocrine regulatory arch in the effect of these ligands on HSC differentiation. However, both intra- and paracrine mechanisms can take place, which requires further investigation. The effect of SCF on primitive $CD34^+$ HSC for the maintenance of these cells in the undifferentiated state is realized through SCL transcription factor (basic helix-loop-helix factor SCL). Inhibition of the expression of this gene with a specific antisence abolished the antiapoptotic effect SCF on HSC [35]. Expression of this gene decreased during differentiation of HSC [20]. At the same time, SCF prolongs the expression of SCL gene, thus maintaining survival of bone marrow $CD34^+$ HSC without differentiation [9]. Taking into account the expression of SCF gene in HSC, we can assume the existence of an important mechanism of autocrine regulation of self-maintenance in HSC population consisting in intracellular formation of a whole chain (ligand—receptor—transcription factor) responsible for signal transfer for the realization of self-maintenance of HSC population without appreciable involvement of exogenous growth

factors. It is possible that transcription factor is involved in HSC differentiation. Inhibition of its expression with antisences or GM-CSF promotes differentiation HSC towards the granulocyte-monocyte differon [3]. However, enhanced expression of this gene stimulates differentiation of human $CD34^+$ HSC towards megakaryo- and erythropoiesis [30]. SCL gene can play a key role in the ontogenetic formation of hemopoiesis. In mice with null-mutation in this gene embryonic stem cells were unable to generate hemopoietic colonies and maintain hemopoiesis [58].

HSC also produce other factors (Fit-3 and thrombopoietin) regulating their proliferative activity [30, 57]. These data suggest that HSC population possesses autocrine regulatory mechanisms ensuring autonomic growth of these cells in the absence of exogenous growth factors and protecting them from apoptotic death.

Taking into account that erythropoiesis is the first functionally active hemopoietic differon in the evolution and ontogeny, erythropoietin also can be considered as a candidate intracellular regulatory factor. Treatment of polypotent HSC with oligodeoxynucleotides to erythropoietin and its receptors determines a considerable decrease in mixed (erythroid/nonerythroid) colonies against the background of in mixed nonerythroid colonies. Authors concluded that erythropoietin and its receptors play an autocrine regulatory role in HSC differentiation towards erythropoiesis [26]. Transcription factor DATA-1 plays an important role in signal transfer from the receptor to erythropoietin in HSC and erythroid precursors [25]. It is unknown whether erythropoietin receptors on HSC are related to their proliferation and differentiation. There are data on proliferative response HSC to erythropoietin without predominant erythroid differentiation effect [19].

The same authors using similar experimental techniques demonstrated expression of genes encoding GM-CSF and their receptors in HSC. An assumption was made on the existence of autocrine regulatory mechanisms of HSC differentiation towards granulopoiesis. Antisence nucleotides to G-CSF and M-CSF were ineffective under these experimental conditions [26,49], which probably attests to the absence of expression of these genes in HSC.

There are published data that stem elements can synthesize some other cytokines involved in autocrine regulation of HSC proliferation and differentiation. Analysis of $CD34^+CD45RAloCD71lo$ HSC from the umbilical vein showed that these cells express IL-1β mRNA, IL-1β-converting enzyme mRNA and produce this cytokine protein. Its synthesis is induced by IL-1α, most likely via the autocrine mechanism, because costimulating effect of IL-1β on proliferation of stem elements was demonstrated [74]. SDF-1, a factor of stromal origin, produced similar autocrine effect on $CD34^+$ HSC. This factor produces an antiapoptotic effect and induces cell transition from G_0 to G_1 [36]. At the same time, *in vivo* inhibitory effect of exogenous SDF-1 on primitive HSC was reported [10]. The existence of different mechanisms of signal transfer in case of auto- and paracrine induction can be hypothesized. Production of IL-1Ra in addition to IL-1β, but not IL-1α (not detected in HSC [4]) suggests auto- and intracrine mechanisms in the regulation of activity of some cytokines in HSC.

Of particular interest are the data on the autocrine role of TGF-β_1 in the regulation of HSC activity. Neutralization of intracellular TGF with monoclonal antibodies or specific antisence reduces proliferative activity of human $CD34^+$ against the background of decreased expression of Bcl-2 gene and enhanced expression of p27 (an inhibitor of cyclin-dependent kinase) and differentiation-related transcription factors PU-1 (specific for differentiation towards granulocytes/monocytes) and GATA-1 (specific for erythroid/megakaryocyte differentiation pathway) [52]. It is possible that in mechanisms of paracrine regulation TGF-β_1 acts as an inhibitor of HSC proliferation suppressing cell transition from G_0/G_1 to S phase of the cell cycle and as a factor decreasing HSC survival [22]. At the same time, in case of autocrine mechanism TGF acts as a factor prolonging proliferative activity of HSC probably via induction of Bcl-2 gene and by impairing their readiness to the differentiation processes. It cannot be excluded that c-Kit-receptor ligand and TGF-β can produce additive or synergistic effects via autocrine mechanisms preserving the proliferative potential of HSC.

Even minimum changes in external appearance of HSC are associated with considerable changes in gene expression. The nearest differentiation of HSC (from $CD34^+CD38^-$ to $CD34^+CD38^+$) is accompanied by enhancement of c-myc and IL-3Rβc gene expression and suppression of TGF-β, G-CSFR, gp130, c-fos, c-jun, and Id gene expression. Expression of some genes can determine the sensitivity of HSC to regulatory factors. It was found that intensive expression of c-fos in primitive HSC is associated with inhibition of cell cycle progression and relative resistance of HSC to growth factors [46]. Reduced expression of TGF-β gene during the studied differentiation is interpreted from the viewpoint of necessary exit of HSC from G_0 phase of the cell cycle [45]. $CD34^+CD38^-$ HSC differ from $CD34^+CD38^+$ cells by considerably increased content of ABCG2-transporter removing some dyes from the cell. This confirms the important role of this gene in the physiology of early HSC subpopulation [61].

Migration of HSC into the circulation under the effect of some regulatory factors is an important property of these cells. There are data on the expression of MIP-1α, MIP-1β, RANTES, SDF-1 (stromal cell-derived factor 1) mRNA and production of the corresponding proteins, which is probably related to enhanced migration of HSC with subsequent activation of hemopoiesis. In $CD34^+$ cells CCR5 chemokine receptor is located intracellulary. It was assumed that this is associated with production of these chemokines by HSC and their subsequent internalization with the receptor [40]. Human $CD34^+$ can also produce MIP-1α protein. Taking into account the inhibitory effect of this chemokine on HSC proliferation we can assume an autocrine negative feed-back mechanism. Autocrine secretion of MIP-1α can explain the absence of the inhibitory effect of its exogenous analogue on proliferative activity of HSC. It was shown that production of chemokines by $CD34^+$ cells and expression of the corresponding receptors on cells increase under the effect of IFN-γ. Cytokines IFN-α, IFN-β, TNF-α, TNF-β, and IL-IL-1α produced no such effects [40]. Hence, HSC via production of chemokines can autonomously interact with other cells, stimulate angiogenesis, and regulate migration and adhesion. The appearance of ectopic hemopoietic foci during stress can be associated with the expression of the above factors in HSC. This assumption is confirmed by the fact that HSC produce VEGF factor (vascular endothelium growth factor) directly regulating the formation of circulatory bed [30].

Migration of HSC is determined by the presence or absence of adhesion molecules on cell membranes. Membrane LFA-1 (leukocyte function associated antigen 1) is not expressed on mouse primitive HSC obtained from the bone marrow or peripheral blood after injection of mobilizing cytokines. Function of HSC was evaluated by colony-forming capacity and radioprotective effect. It was concluded that LFA-1-negative HSC are more primitive compared to LFA-1-positive cells [56]. Authors assume the involvement of other cells (*e.g.* neutrophils) in the mechanisms of HSC mobilization from the bone marrow to the periphery. It was shown that expression LFA-1 gene in mouse and human HSC *in vitro* during culturing determines high proliferative potential of HSC and has no effect on the formation of large colonies on day 14 in culture [69]. Expression of this adhesion molecule on HSC results from a default (the absence of negative signal from stromal cells) [69]. This is probably a first manifestation of future intracellular differentiation processes in HSC. Expression of adhesion molecules is probably a dynamic process and determines the behavior of HSC in hemopoietic organs. Successive expression of integrins LFA-1, VLA-4, VLA-5, and E and P selectin ligands on HSC is putative inductors of HSC migration through bone marrow microenvironment to "HSC niches". Treatment with antibodies to VLA-4 and VLA-5 integrins abolished the formation of 12-day colonies of hemopoietic HSC in mouse spleen [50].

Apoptosis determining natural non-inflammatory and non-necrotic cell death is an important mechanism maintaining quantitative and qualitative balance in all cell populations. Similarly to many other cell population, the mechanism of apoptosis takes place in HSC. Most likely, human $CD34^+CD38^-$ HSC are resistant to Fas-mediated apoptosis, despite the expression (though minor) of Fas-antigen and caspase 8 in these cells. After the interaction with FasL, *in vitro* colony-forming capacity and *in vivo* grafting potential tended to increase. The authors explain this property of HSC by intensive production of FLIP protein, an inhibitor of caspase 8 and apoptosis [33]. It was shown that ~50% HSC express cytoplasmic antiapoptotic Bcl-2 molecules [67]. More that 94% HSC ($CD34^+lin\text{-}CD38^-$) express antiapoptotic Bcl-xL gene, and only ~4% express Bcl-2. This confirms the important role of Bcl-xL gene in long-term survival of HSC population [48]. Expression of this gene increases during combined exposure of HSC to erythropoietin and stem cell factor, as well as during erythroid differentiation of HSC [31]. Expression of Bax gene was suppressed under these conditions. Combined action of stem cell factor and G-CSF on these HSC suppressed the expression of Bcl-xL against the background of unchanged expression of Bax gene. Bcl-xL gene is related to survival of erythroid precursors, rather than to their differentiation from HSC. The participation of Bcl-xL gene in mechanisms of survival in population of HSC and erythroid, but not granuloid precursors, probably attests to the readiness of HSC for erythroid differentiation under different stress conditions. Bcl-2 gene probably plays an antiapoptotic role in HSC. In transgenic mice with enhanced expression of Bcl-2 the number of HSC in the bone marrow and their repopulation potential were increased [16,17]. However, enhanced expression of this gene cannot prevent HSC death in a serum-free medium; realization of the effect of this gene requires the presence of Kit-ligand (steel factor). Under experimental conditions, increased proliferative activity of HSC primarily resulted in intensification of differentiation to oligopotent precursors, but not intensified self-maintenance processes [16,17].

Thus, HSC possess a variety of regulatory mechanisms largely determining the main functional activities of these cells: proliferation, self-maintenance, migration, and differentiation. They can be divided into three groups: transcription factors, specific receptors for regulatory molecules, regulatory molecules

(cytokines, chemokines, *etc.*). Undoubtedly, there are close relationships between these molecules. These relationships can be realized inside the cells not only by classical pathway (inducer, receptor, transcription factor, gene expression), but also through interaction between these molecules. It cannot be excluded that these interactions should be divided into homologous (*e.g.* between transcription factors, receptors, cytokines) and heterologous (between transcription factors and cytokines). Changes in the molecules, neutralization or activation, modulate HSC sensitivity to exo- and endogenous regulatory effects.

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