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Precursor cells in the hematopoietic stromal microenvironment

Joseph L. Chertkov (†), Olga A. Gurevitch, Gennadiy A. Udalov (†), Irina N. Shipounova, Nina J. Drize

National Research Center for Hematology, Moscow, Russia

Correspondence: Nina Drize, National Research Center for Hematology, Russian Academy of Medical Science, Novozykovsky pr. 4a, 125167 Moscow, Russia.
Tel.: +7 495 612 3521, Fax: +7 495 614 9269, E-mail: ndrize@yandex.ru

Abstract

The manuscript summarizes the works of Prof. Joseph Chertkov that are dedicated to precursor cells in the hematopoietic stromal microenvironment. Unique functional analysis was used in these investigations. The properties of stem cells in the hematopoietic microenvironment such as self-renewal capacity and the ability to differentiate into all stromal lineages are described. The hierarchical structure of stromal precursor cells compartment is proposed. Some elements of the regulatory pathways of stromal precursor cells are described. This compilation reflects the importance of Prof. Chertkov's contribution to the investigation of stromal precursor cells and hematopoiesis.

Keywords: stromal microenvironment, mesenchymal stem cells, inducible stromal precursor cells, ectopic hematopoietic foci, long-term bone marrow culture

Introduction

Prof. J. L. Chertkov (1927–2009) devoted most of his efforts to understanding the mechanisms of hematopoiesis. He was interested in the developmental fate of hematopoietic stem cells (HSC), and the result of his investigations became the theory of clonal succession of HSC, which was published in several papers [1-4]. The data showed that long-term hematopoiesis is maintained by a large number of simultaneously functioning small, short-lived (1 to 3 months) clones that usually grow locally with little or no dispersion between different regions of the hematopoietic system. Only 10% of clones are long-lived and can function during the whole life of the animal. Furthermore, clones that disappear are never detected again. The data suggests that normal hematopoiesis is supported by the sequential recruitment of marrow repopulating cells into a differentiation mode.

In the mid 1970s, together with A. J. Friedenstein, Joseph Chertkov laid the foundation for experimentation into the differences between HSC and precursor cells in the hematopoietic microenvironment. He postulated that an understanding of the interrelationship between the stem cells of hematopoiesis and regulatory stromal microenvironment is necessary for an investigation of the process of hematopoiesis. To analyze the stromal precursor cells J. L. Chertkov used a functional assay where the microenvironment is addressed as the territory where hematopoiesis takes place and therefore could be estimated by the number of hematopoietic cells maintained on it. The method of ectopic hematopoietic foci formation provides a separate hematopoietic territory built de novo via special stromal precursor cells. Cells capable of transferring the hematopoietic microenvironment were referred to by Chertkov as hematopoietic microenvironment-transferring units (HMTU) [5].

In 1991 A. Caplan defined stem cells capable of giving rise to skeletal tissues – cartilage, bone, tendon, ligament, marrow stroma, and connective tissue – as mesenchymal stem cells (MSC) [6]. The term MSC was used in the fields of cytotherapy and tissue engineering widely and not always correctly; therefore the International Society of Cellular Therapy postulated the use of the term MSC only for cells that fulfilled the stem cell criteria: multipotentiality and self-renewal [7]. All works of Prof. Chertkov clearly demonstrate that HMTU and MSC are synonyms. Therefore we will use the term MSC for cells described by J. L. Chertkov as HMTU.

The compilation of the works by J. L. Chertkov characterizes MSCs both quantitatively and qualitatively, based on their functional properties. MSCs were shown to have a high proliferative potential, to be able to develop multilineage progeny, and form a fully functional hematopoietic microenvironment. The compartment of stromal precursor cells was shown to have a hierarchical

structure, and inducible precursor cells were characterized. The radiosensitivity of MSCs and their progeny was estimated and it was then possible to calculate the direct number of MSC in the murine femur.

Materials and Methods

Mice

Female and male C57BL/6 (B6), CBA, CBAT6T6, (CBAxC57BL/6) F_1 hybrid (CB), and (CBAT6T6xC57BL/6) F_1 hybrid (CBT) mice 8–25 wks of age at the beginning of the study were used. Care was taken that the groups to be compared originated from the same batch of animals housed under the same conditions.

Irradiation of mice

In some of the experiments the recipient mice were irradiated with 400 or 700 rad from a ^{137}Cs IPK irradiator 3 to 4 hrs before bone marrow (BM) implantation. Both doses produced the same effect on the size of the ectopic foci. The irradiator consisted of four ^{137}Cs sources set in a quadrilateral arrangement about the site of exposure.

In order to obtain blood sera containing stroma-stimulating activity, the mice were irradiated with 6–12 Gy (in the latter case the protective dose of BM cells was injected i.v.).

Bone marrow irradiation

In the case of an in vivo irradiation the mice were irradiated and sacrificed immediately thereafter. The femurs were removed and stored on ice until transplantation. For in vitro irradiation the femurs were exposed either to γ-rays from an IPK irradiator with the absorbed dose rate of 500 rad/min or to fast neutrons generated by the Obninsk BR-10 research reactor. The mean energy of fast neutrons was 0.85MeV, the power of the tissue Kerma 128 rad/min, and the ratio of neutron to γ-ray doses at the point of exposure of the bones was approximately 20:1. The γ-ray component was ignored in calculating the dose-response curves, and all neutron doses represent the rad dose of the neutron component. In all experiments, the period between bone resection and the implantation of the bone marrow did not exceed 5 hrs. The sequence of implantation of femurs exposed to various doses was always randomized.

Chimeras

The mice were exposed to 12–13 Gy and reconstituted with syngeneic or allogeneic BM in doses indicated in the corresponding part of the text. In general, 1/3–1/4 of the femoral equivalent was injected (standard chimeras). Secondary chimeras were obtained when irradiated recipients were reconstituted with hematopoietic cells of standard chimeras; the cells were collected no earlier than 2 months after the creation of the standard chimeras. Tertiary chimeras were obtained when the irradiated recipients were reconstituted with hematopoietic cells of secondary chimeras; the cells were also collected no earlier than 2 months after the creation of the secondary chimeras. Double chimeras were obtained when the standard chimeras were exposed to a dose of 12–13 Gy 2–5 months after creation of the chimera, and reconstituted with hematopoietic cells of normal mice. The stromal precursors were studied 3–9 months after creation of the chimeras.

Bone marrow or adherent cell layer implantation

Implantation was performed under the renal capsule of anesthetized mice. The femurs were freed of muscle, the epiphyses cut away, and the bones stored on ice until used. The BM was pressed out of the femur with a stylet or thick needle with a blunt end. In anesthetized mice a small tear was made in the renal capsule and a bone marrow plug or adherent cell layer (ACL) from long-term bone marrow culture placed under it with a small spatula. The ACL was removed from the flask bottom with a rubber policeman and implanted under the renal capsule without conversion to a single-cell suspension. In cases of ectopic foci reimplantation the whole focus was removed from the kidney and implanted under the renal capsule of the recipient. In cases where suspended BM was implanted, the 0.5 ml of suspension (made by repeated passage through a 23-gauge needle) containing $1–2x10^7$ cells was precipitated via centrifugation onto a Millipore HA filter (0.45 micron). The filter was folded so that the cells were inside it and then was transplanted under the renal capsule of the recipient mice. The size of the foci produced was determined after 1–1.5 months by counting the number of nucleated hematopoietic cells in them. The ossicle containing BM was removed from the kidney, and the cells were scraped off the bone with a scalpel into medium 199 or α-MEM and prepared as a single cell suspension by passing it repeatedly through a syringe fitted with a 21-gauge needle. In some experiments the cellularity of the foci was determined in the pool of all foci in each group; consequently, the standard error cannot be calculated. In general the error in such experiments was about 20%.

Bone marrow ablation

After anesthesia, a small incision was made over the knee joint, and the medullary cavity of the femur was entered and curetted using a dental root-canal broach. This was followed by the insertion of a 23-gauge needle into the medullary cavity of the curetted femur, which was then irrigated vigorously with 1 ml of medium 199.

Determination of the proliferative activity of stromal precursors in vivo

The S-phase specific cytostatic compound methotrexate (MTX) was injected intraperitoneally in a single dose of 0.25 mg/g. This dose of MTX was lethal and therefore 4 hrs after its injection the bone marrow or ectopic site was transferred into a normal recipient.

Long-term bone marrow culture

The marrow cells or the cells of an ectopic hematopoietic focus were cultivated by the method described by Dexter et al. [8]. The cells were flushed out with 10 ml of complete medium into a $25cm^2$ flask without converting them to a single-cell suspension. In the case of cultivation of suspended BM cells, 1 femur was suspended by repeated passage through a 21-gauge needle and then seeded onto the $25cm^2$ flask. When cultivated in a 24-well plate, 2 femurs were explanted per plate, also without conversion

into a single-cell suspension. Fisher medium supplemented with L-glutamine, antibiotics (all Flow Labs), 25% serum (2:1, horse: fetal calf sera, Gibco and Flow Labs) and 10^{-6}M hydrocortisone sodium hemisuccinate (Sigma) were used. The culture was kept at 33^0C and 5% of CO_2 with weekly replacement of 50% of the medium.

The "wound" was performed by scraping 1/2of the adherent cell layer (ACL) with the rubber policeman.

Determination of the proliferative activity of stromal precursors in vitro

Hydroxyurea was added to a long-term bone marrow culture (LT-BMC) at the concentration of 13mM (1mg/ml) for periods from 2 hr to 7 days. To stop the function of hydroxyurea the ACLs were washed 3 times with 5 ml of medium 199 with 2% of FCS.

Cytokine treatment

Cytokines (recombinant rat SCF (Amgen) and recombinant human G-CSF (Neupogen 48, Amgen)) were dissolved into the 0.9% NaCl solution with 0.1% of BSA and injected once a day under the skin for 6, 10, or 17 days. G-CSF was used at the concentration of 250 mkg/kg, and SCF at 34 mkg/kg. The control group was injected with 0.9% NaCl solution with 0.1% of BSA only. Twenty hours or 1 month after the last injection, BM from the femurs of the control and cytokine-treated mice was implanted under the renal capsule of the syngeneic mice. In order to define the effect of G-CSF on foci formation the mice were implanted with the syngeneic BM 1 day before beginning the G-CSF courses, which lasted 10 or 17 days.

Sera from irradiated mice

Blood was obtained from the femoral vein not earlier than 1 week after the irradiation. After the clot retraction sera were centrifuged (3000 rpm), supernatant was sterilized by filtration through 0.22 μm filters.

Analysis of various organs of irradiated mice for stroma-stimulating activity

Bone marrow, thymus, bones, liver, and spleen of irradiated mice were implanted into intact mice under the skin or renal capsule. Suspended spleen cells were injected intravenously to the mice previously treated with heparin (50 U/mouse). Intact BM was implanted simultaneously under the renal capsule of these mice.

Karyotype analysis

The origin of the hematopoietic cells in the focus was determined according to the presence or absence of Y-chromosomes, using the G-banding technique.

Histology

The kidneys were removed and fixed in Carnoy's solution, decalcified, embedded in paraffin and cut into series of 5 μm sections. The preparations were stained with Pappenheim, Giemsa, and hematoxylin-eosin stains.

Statistics

The radiosensitivity curves were fitted to the data via linear regression analysis, from which the D_0s, standard errors, and extrapolation numbers were calculated. The concentration of MSC in the femur was calculated using Poisson's distribution. When not otherwise noted, the data were analyzed with Student's t-test.

Results and Discussion

Methods of in vitro and in vivo mesenchymal stem cells analysis

In the case of bone marrow (BM) implantation under the renal capsule of the syngeneic animal, the hematopoietic cells leave the graft, whereas the stromal precursors form the new hematopoietic stroma, which is then repopulated by host cells [9,10]. The same processes take place after implantation of an adherent cell layer from 3–4 wk bone marrow cultures. The beginning of vessel formation was evident by 12 h after implantation. The blood supply to the implant was established 24–48 h after implantation. Characteristic connective tissue lacunae form, and clusters of connective tissue cells were seen from day 4–5, as well as strands of fibroblasts and sometimes the beginning of cartilage development, which was replaced by osteogenesis. By day 6 the implant on the side toward the kidney contained many dead cells with pyknotic nuclei, many erythrocytes, and colonies of hemopoietic cells with blasts cells in the center. On the top and sides of the implant cancellous bone formation was observed. Within the foci there were individual osteoblasts in broad cavities with developing bone trabeculae around them. Osteogenesis was very intensive and 1–2 days later large areas of de novo formed bone could be observed. After 11 days, the typical bone marrow structures such as sinusoids, adipocytes, and areas of hemopoietic cells of different lines of differentiation were represented, and by the end of the second week a well-developed ectopic hemopoietic foci was created [11].

Polymorphic hemopoiesis is maintained for many weeks in long-term cultures of adult mouse bone marrow, and all the main categories of hematopoietic cell precursors, hematopoietic stem cells among others, are identified. Such cultures are characterized by the formation of an adherent cell layer (ACL) of a complex composition containing fibroblastoid cells, giant adipocytes, endothelial cells, and macrophages. The ACL acts as the hematopoietic microenvironment necessary for support of proliferation and differentiation of the hematopoietic cells. It is natural, therefore, to assume that the microenvironment is created by the same precursors as in culture and in vivo, i.e., MSCs capable of transferring the hematopoietic environment and creating ectopic hematopoietic foci upon transplantation.

The transplantation of ACLs from 3–4-week-old cultures of syngeneic animals led to the creation of ectopic foci with the size of the foci formed from a fresh bone marrow plug ($5–15x10^6$ nucleated cells). Hematopoietic cells of all differentiation lineages were seen in the foci. The relative CFU-S content was the same as that in the bone marrow (11.4±3.4 per 10^5 cells) and did not differ from that in the foci produced from bone marrow plug [12]. Thus, cultivation in the long-term culture did not affect stromal precursor dramatically.

The importance of intercellular contacts for appropriate MSC function

Importantly, the bone marrow plug (or cells from long-term bone marrow culture) should be implanted under the renal capsule as a whole, while carefully avoiding converting it into a suspension. Implantation of cells as a suspension under the renal capsule means that no ectopic hematopoietic foci form [13] (for example with cells from long-term bone marrow cultures see Table 1).
Simultaneous i.v. injection of bone marrow cells to donors did not affect the size of the foci formed from either irradiated donors, or donors with previously curetted bone marrow; meaning that mesenchymal stem cell (MSCs) numbers in the femurs were not affected by the injected cells [14].

One of the most important factors providing for the formation of a full-grown hematopoietic microenvironment in vitro is the explantation of the BM as a whole, or in fragments, but not as a suspension [8]. When the BM is seeded as a suspension, cells do not form the complex stromal layer; on the contrary, cells form a thin monolayer with fibroblast colonies. No hematopoiesis is observed in such layers. Therefore the dissociation of bone marrow cells changes their differentiation potential. Stromal progenitors do not fulfill the variety of cellular differentiations leading to the formation of complex structures of a hematopoietic microenvironment in culture. Thus, the intercellular connections are of most importance for the preserving of the MSC's features.

When explanted in fragments, bone marrow stromal cells, initially occupying a tiny part of the cultivation flask, built hematopoietic stromal structures on the whole flask surface. During this process they do not lose (or constantly renew) essential intercellular contacts. In order to find out whether this ability is present in the cells from a completely formed cell layer a "wound" was inflicted on the 3-week-old ACL. One week later fibroblastoid cells were observed only rarely on the wound site. No complex ACL was formed. Completely different results were obtained when the "wound" was inflicted on a 1-week-old cell layer, it being in the formation process. One week later the wound site was covered with typical for ACL stromal cells so tightly that it was hardly definable. The ACL regenerated not only morphologically but also functionally as was demonstrated by the ectopic foci formation (Table 2).

№ of experiment	Method of implantation	Culture age, weeks	Number of cultures implanted	Foci size, x10^6 cells
1	Suspension	7	4	0
2	Suspension	5	2	0
	Fragments	5	2	9.3
3	Suspension	4	2	0
	Fragments	4	2	8.0

Table 1: Transfer of hematopoietic microenvironment via cultures of bone marrow either in fragments or in cell suspension

ACL	Number of cultures	Foci size, x10^6 cells
Intact Part	4	3.8
Regenerated part	4	1.2

Table 2: Size of the foci formed by intact 3-week-old or regenerated ACL (ACL was wounded after 1 week of cultivation)

Time after irradiation, months	Number of chimeras tested	Recipient of implanted chimeric bone marrow	Foci formed/ number of implants
6	3	B6	0/2
		CBF	4/4
12	18	B6	0/16
		CBF	20/20

Table 3: Discriminant analysis of hemopoietic stromal progenitor origin in B6-in-CBF chimeras

Donor	Recipient	Cells per focus, x10^6	Donor/recipient metaphases	Foci formed/ number of implants
B6 female	B6 male	24.4	0/100	2/2
B6 female	B6 male	15.7	0/45	1/2
B6 female	B6 male	10.4	0/40	1/2
B6 female	B6 male	13.5	0/60	1/2
B6 female	B6 male	5.8	0/7	1/2

Table 4: The origin of hematopoietic cells in ectopic hemopoietic foci produced by adherent cell layer from long-term bone marrow culture

Thus, the intercellular contacts are of highest importance for the stromal progenitors' differentiation during the processes of building the hematopoietic microenvironment. Stromal progenitor cells are able to keep the intercellular contacts while forming the microenvironment in vitro, and lose this ability when the functional adherent cell layer is formed [13].

The origin of stromal and hematopoietic cells in chimeras and the ectopic foci

Discriminant analysis showed that only MSCs of recipient origin are present in chimera bone marrow 6 and 12 months after irradiation and injection of hematopoietic cells (3–5x10^6) (Table 3).

Chimera marrow implanted to non-irradiated mice of the donor strain (B6) was always rejected, and the hematopoietic microenvironment could only be transferred to the recipient strain (CBF) [14]. This means, firstly, that stromal cells in the ectopic hematopoietic foci are of BM donor origin, and secondly, that BM stromal cells injected intravenously for the reconstitution of hematopoiesis after irradiation did not engraft bone marrow stroma of chimeras.

The hematopoietic cells in the ectopic foci were only of recipient origin, as was shown after implantation of adherent cells from B6 female bone marrow cultures under the renal capsule of B6 males (Table 4). The origin of hematopoietic cells in the focus was determined accor-

ding to the presence or absence of a Y-chromosome [11].

The results show that stromal progenitors capable of hematopoietic microenvironment transfer cannot be transplanted i.v., and do not take part in MSC regeneration after irradiation. On implantation of BM of intact mice or adherent cell layer from bone marrow cultures under the renal capsule, ectopic hematopoietic foci form, in which the hematopoietic cells belong to the recipient while the stroma is of donor origin.

In order to investigate the origin of stromal cells in ACL, LTBMCs had been established from B6-in-CBF1chimeras, and when stable ACLs had been formed they were carefully scraped as a whole and implanted under the renal capsule in both B6 and CBF1 recipients. This discriminant analysis showed that ACL implantation produced ectopic foci only in the recipient line (CBF1) (Table 5).

Thus, MSCs in the ACLs from long-term bone marrow cultures of chimeras are only of recipient origin [14].

Linear interdependency between the foci size and amount of MSCs implanted

Various amounts of medullary tissue ranging from 1/4 to 4 femoral bone marrow plugs were transplanted and the hematopoietic cells were counted in the foci formed 1 month later. The results are shown in Table 6, where it can be seen that despite the small number of implants (3–7), the number of nucleated cells on the whole showed a linear relation with the size of the implanted bone marrow fragment. The correlation coefficient (r) was 0.97±0.014 and the extrapolation number $4.68x10^{-5}$ [5].

In summary, stromal cells implanted under the renal capsule of syngeneic animal form a hematopoietic microenvironment only if MSCs are preserved among them, and the size of these hematopoietic foci depends solely on the number of MSC among implanted stromal cells. These results allow MSC to be studied on this model.

The size of ectopic foci is proportional to the amount of ACL implanted. ACL taken from the half of the flask bottom produces a focus that is approximately half that formed by ACL collected from the whole surface of the culture flask: $35.6x10^6$ and $58.1x10^6$ nucleated cells, respectively (correlation coefficient is 0.996±0.005) [12].

When transplanted under the renal capsule of a syngeneic recipient, ACL of cultures of a single femur creates a focus approximately the size of a focus formed in implantation of bone marrow freshly isolated from a single femur. This coincidence suggests that the content of stroma precursors in the culture corresponds to the explanted bone marrow dose but not to other factors, for instance, the surface of the flask bottom. In view of this, the size of foci produced by ACL from 4–6-week-old cultures of 1/2, 1 and 2 femurs was studied (Table 7).

The size of the foci was linearly associated with the dose of the implanted bone marrow (correlation coefficient 0.999±0.001) [12].

Kinetics of stromal precursor cell proliferation in vitro and during ectopic foci formation

The time-course of the stromal precursor repopulation during the process of a creating a site of ectopic hematopoiesis was also studied. In the first 6 hrs after implantation the number of transplantable stromal precursors reduced appreciably, reaching the nadir by the end of the first day (about 20% of the number implanted). Thereafter, there was a phase of regeneration and stromal precursors recovered up to the initial level in 3 weeks. The sensitivity of the stromal precursors to the cytostatics is in good agreement with such kinetics. Normally the stromal precursors do not actually show proliferative activity, which is seen from their

Time after irradiation, months	Number of chimeras tested	Recipient of implanted ACL	Foci formed/ number of implants
12	4	B6	0/4
		CBF1	4/4

Table 5: Discriminant analysis of hematopoietic stromal progenitors originating in adherent cell layer (ACL) of long-term bone marrow cultures of B6-in-CBF1 chimeras

Experiment №	Size of implant (femoral marrow plug equivalent)	Number of implants	Hematopoietic cells/ focus ($x10^6$)
1	¼	5	2.5
	1	5	4.7
2	¼	5	2.6
	½	7	3.4
	1	6	10.0
3	¼	3	3.8
	1	4	8.1
4	1	5	16.5
	2	4	30.3
	4	3	68.0
5	¼	3	68.0
	½	10	7.9
	1	9	9.8

Table 6: The influence of ectopic marrow implant size on hematopoietic cell number in the foci formed

Bone marrow plated per flask (femur equivalent)	Foci formed/Number of implants	Ectopic foci size, $x10^6$ cells
½	5/7	1.7
1	10/13	4
2	9/10	8.3

Table 7: Correlation between bone marrow dose plated in LTBMC and the size of the ectopic foci formed

insensitivity to MTX. Twenty-four hours after implantation their proliferative activity remains low. During the next 24 hrs the precursors are triggered into the cell cycle synchronously. At this time up to two-thirds of the stromal precursors are killed by the cytostatics. High sensitivity to the cytostatics is observed at 3–4 and 9–10 days after implantation, but not 5–6 days after. It is not clear whether these fluctuations are incidental, or if they are related to the movement of the partially synchronized cell population through the cell cycle. Three weeks later, i.e., when the number of stromal precursors had recovered, their proliferative activity decreased to the initial low level, and remained on that level [15].

Stromal precursors also proliferate during the formation of the adherent cell layer in the LTBMC. Considering the fact that hydroxyurea affects precursors from day 2 until day 11, it seems that these cells change their proliferative status slowly, for more than 24 hours. Thus, both the implantation of BM in vivo, leading to the building of a new hematopoietic microenvironment and hematopoietic foci formation, and the explantation of BM in vitro, leading to the building of a hematopoietic microenvironment in the form of an adherent cell layer in LTBMC, are accompanied by identical changes in the proliferative status of stromal precursors. In the first 24 hours after the transfer they remain at mitotic rest, then, during the next 2 days they mobilize into the cell cycle substantially and synchronically. For the next 2 weeks they are highly active in proliferation. Afterwards, despite continuous growth of ectopic foci or ACLs, the stromal precursors do not proliferate. This is reflected in the absence of increase in the number of stromal precursors both in vivo and in vitro after 3 weeks of formation of the microenvironment.

Characteristics of stromal progenitors in vivo and in vitro

De novo formation of a stromal microenvironment after the implantation of MSC under the renal capsule

In bone marrow implantation the hematopoietic cells leave the graft, whereas the stromal precursors form a new hematopoietic stroma, which is then repopulated by host cells. The cellularity of the hematopoietic focus is proportional to the initial implant size, i.e., to the content of the stromal precursors in it. On retransplantation of the intact ectopic site, hemopoietic cells again leave the implant, as they do after the primary implantation of the bone marrow plug. Twenty-four hours after retransplantation no more than about 3% of the CFU-S remain in the focus (Table 8) [15].

The replacement of the hematopoietic cells by the recipient cells in the retransplanted focus was also confirmed karyologically [16]. Hence, it appears that when the formed site of ectopic hematopoiesis is retransplanted, the hematopoietic microenvironment is created de novo.

Time after retransplantation, days	Number of implants	Cellularity of ectopic foci, x10^6	CFU-S per ectopic foci
Before	5	12.1	3842±545
1	6	3.3	107±16
4	6	1.8	148±18
7	5	4.5	641±90
10	6	7.9	1501±329

Table 8: Cellularity and CFU-S content of an ectopic site of hematopoiesis as a function of time after retransplantation

Self-renewal ability of MSC

The results permitted the study of the capacity of MSCs for repeated formation of ectopic foci. Nine passages failed to produce any reduction in size of the newly formed hematopoietic foci (Table 9).

Transfer number	Cells in the foci, x106
1	12.5
2	17.2
3	20.6
4	21.3
5	22.9
6	19.8
7	35.2
8	24.7
9	11.5

Table 9: Cellularity of ectopic bone marrow foci on repeated transplantation

During the serial transfer of the ectopic hematopoietic tissue without ossicles, a complete loss of the ability to form the hematopoietic focus was already apparent at the third passage. In this case no more than half of the stromal precursors remained on the ossicle (when the bone marrow is pressed out of the femur only 10–15% of the stroma precursors remain on the bone), which was verified by separate implantation of the ossicle and hematopoietic tissue from focus [15].

The self-maintenance ability of the stromal precursors was also studied in a model in which the medullary cavity was repeatedly curetted. Four successive curettages were carried out, and in each over 90% of the stromal precursors were washed out of the femur. After each curettage, the complement of stromal precursors recovered over 1–1.5 months up to 50–60% of the initial and after the fourth curettage up to 20%. Subsequent curettages proved impossible because the whole medullary cavity was filled with newly formed bone [15].

Experiment №	Intermediate recipients		Final recipients	
	Foci/number of implants	Cellularity, x10^6	Foci/number of implants	Cellularity, x10^6
1	4/4	12.1	4/4	1.8
2	3/4	2.9	3/3	0.6

Table 10: Self-maintenance ability of hematopoietic stromal precursors from long-term bone marrow cultures

The capacity for self-maintenance of stromal precursors from cultures was studied by repeated transfer of ectopic hemopoietic foci created by them in intermediate to final recipients (Table 10).

The self-maintenance of MSCs from LTBMC proved to be low and the size of the secondary foci was 10–15% of that of foci in the intermediate recipients [11]. Thus, MSCs are maintained in LTBMC and are capable of creating a hematopoietic microenvironment on transplantation. However, self-maintenance of these precursors from LTBMC is essentially diminished.

The data obtained have demonstrated a high ability of self-maintenance of the cells transferring the hematopoietic microenvironment. Twenty-four hours after BM implantation about 10% of the stromal precursors survive. The population of the precursors in the femur is reduced to approximately the same degree after bone marrow curettage. During regeneration the stromal precursors increase to 60–100% of the initial level, hence they must undergo three or four mitoses. This is consistent with the rise in their sensitivity to S-phase specific cytostatics for the first 2 weeks after implantation. Taking into consideration that on the serial transfer of hematopoietic tissue without ossicles or on serial curettage three or four cycles of regeneration are possible, the stromal precursors are able to undergo no less than 10–12 mitoses. This value is rather underestimated since calculating the loss of precursors for differentiation was not taken into account. The high self-maintenance ability, on the one hand, and the ability to form a fully differentiated bone marrow stromal tissue on the other, suggests that the cells transferring the hematopoietic microenvironment are true mesenchymal stem cells.

Radiosensitivity of MSCs

The hematopoietic stroma function of the femoral bone marrow exposed in vitro to 500 to 2700 rad of γ-rays was also studied, using the ectopic foci formation method. Irradiation of bones with 500 rad caused no noticeable damage to the MSCs' ability to form a hematopoietic microenvironment. Higher doses produced an exponential decrease in MSCs. The D_0 estimated from linear regression (regression equation: log survival=-0.000977x+0.7200) of this portion of the curve is 444±5 rad and the extrapolation number (n) is 5.2. The results for in vitro neutron irradiation of bone marrow agreed (regression equation: log survival=-0.002697x+0.1506). A small shoulder was evident followed by an exponential survival having D_0 and n of 161±19 rad and 1.4, respectively [5].

The radiosensitivity of MSCs from 5-week-old LTBMC seemed very similar to that of non-cultivated ones (regression equation: log survival=-0.089x+0.3744; D_0 was 486±15 rad and n was 2.4) [17].

When high doses of irradiation were used, implantation proved unsuccessful in some cases and no ossicles with hematopoiesis were formed. One may assume that not a single MSC is preserved in such implants. The independent and random character of radiation damage of cells suggests that the existence or nonexistence of MSCs in the implant is governed by Poisson's distribution. In this case the data on the proportion of transplant failures (P_0) allow the mean MSC content in the implant (x) to be calculated using the equation $x=- \ln P_0$. The total MSC content in the femoral bone marrow can be found by taking into account the fraction that survived exposure to the given dose. These data are shown in Table 11 [5] where it is seen that the results were quite consistent on the whole.

The mean number of MSCs calculated from the proportion of transplant failures after high doses of irradiation was 52.3±11.6 per femoral bone marrow plug.

The results show that MSCs are much more radioresistant than hematopoietic stem cells, for which D0 is about 100 rad. The second feature of MSCs is their marked capability to recover from sublethal γ-ray damage, which is characterized by a extrapolation number (5.2). In the case of neutron irradiation the extrapolation number is 1.4, which is evidence that MSCs are incapable of recovery from sublethal neutron-induced damage [5].

Despite their high radioresistance MSCs were still sensitive to irradiation. After lethal irradiation and syngeneic bone marrow transplantation of CBF1 mice, the MSCs were reduced to 1/5 of the initial level and slowly regenerated to subnormal level in 6 months. The number of bone marrow cells used for reconstitution of the primary recipient did not affect MSC regeneration (Table 12) [14].

Two to six months after irradiation, the content of stromal progenitors in the mouse femur was, judging from the cellularity of the foci produced by them, 40–50% of the initial level in both groups of mice reconstituted both in minimal pro-

Irradiation dose, rad	Implant failure/ total number of implants	MSC/implant ($- \ln P_0$)	Survival fraction	MSC per femur
2100	6/22	1.3	0.033	39.9
2200	12/20	0.511	0.025	20.3
2200	9/21	0.847	0.013	65.4
2500	7/19	0.999	0.012	86.3
2700	17/20	0.163	0.007	23.3
2700	15/19	0.236	0.003	78.7

Table 11: The effect of g irradiation on the hematopoietic microenvironment transferring MSCs in murine femoral bone marrow

Chimera's characteristics		Ectopic hemopoietic foci produced by femoral marrow plug implantation from reconstituted mice		
Cell injected	Time after reconstitution, months	Foci formed/ number of implants	Foci cellularity, $x10^6$	Ossicle weight, mg
2x105	2	8/8	4.0	1.4
7.4x107	2	8/8	4.8	1.4
2x105	6	8/8	5.5	2.0
7.4x107	6	8/8	5.4	2.1
control	--	12/12	10.7	1.6

Table 12: Hematopoietic stromal precursors in lethally irradiated CBF1 mice reconstituted with different doses of syngeneic bone marrow cells

tective dose of marrow cells ($2x10^5$) and with a hundredfold dose. Thus, MSCs could be affected by high doses of irradiation and in such cases they are not able to regenerate completely.

Influence of the quality of hematopoiesis on MSCs' proliferative potential

MSCs were compared in chimeras and double chimeras. Hematopoietic foci formed in standard recipients via BM from chimeras were approximately 2 times smaller when compared with foci formed from BM of non-irradiated mice, and 2 times bigger compared to foci formed from BM from double chimeras. These data confirm that stromal precursors are radiosensitive and unable to recover from radiation damage completely. Interestingly, secondary and tertiary chimeras' BM formed the same small ectopic foci as double chimeras did, while the dose of irradiation affected the stroma of secondary and tertiary chimeras was 2 times lower than of double chimeras [18]. When BM from all types of chimeras tested were explanted in LTBMC, and after 3–4 weeks of cultivation were implanted under the renal capsule of syngeneic mice, foci formed from ACLs from chimeras' BM were 75%, foci formed from ACLs from double chimeras' BM were 20%, and ACLs from secondary and tertiary chimeras were approximately 40% by size from ACLs from non-irradiated mice. The secondary and tertiary chimeras' stromal precursors were irradiated only once while hematopoietic cells had undergone 2 or 3 rounds of intensive proliferation during reconstitution of hematopoiesis. Thus, judging by the grade of irradiation damage, stromal cells from secondary and tertiary chimeras were similar to ordinary chimeras. Nevertheless, secondary and tertiary chimeras' stromal precursors turned out to be affected more deeply than the same cells in ordinary chimeras. The data indicates that the stroma's damage was determined not only by the irradiation dosage but by the quality of hematopoietic cells proliferating on it [18].

Hierarchical organization of the MSC compartment: existence of more mature than MSC-inducible precursor cells

In BM implantation, the ectopic hemopoietic focus is larger in an irradiated recipient than in a non-irradiated one in a dose-dependent manner (Table 13) [19].

In subsequent passages the size of the focus did not increase further (Table 14, compare with Table 9).

Hence, one may conclude that in the irradiated recipient the number of stromal precursors in an ectopic focus does not correspond to the large size of the focus. This was demonstrated more directly by implantation of similar bone marrow fragments into non-irradiated and irradiated (chimeric) recipients, subsequently testing the content of the stromal precursors in the sites formed by their retransplantation to non-irradiated recipients. In these experiments the primary focus formed in chimeras exceeded that in the non-irradiated ones by a factor of 2.2 ($27.3x10^6$ and $12.5x10^6$). The content of the stromal precursors in both was essentially the same since the cellularities of the foci formed on transfer to the non-irradiated recipients were $10.93x10^6$ and $11.83x10^6$, respectively [15]. Stromal precursors from a culture also react to stimulation from the irradiated recipient, the response being much stronger than in implantation of freshly isolated bone marrow: the foci were 5–7 times larger in the irradiated recipients than in the non-irradiated ones ($35.1\pm8x10^6$ versus $5.3\pm1.2x10^6$) (Table 15) [11].

Similar results were obtained when LTBMC were established from different amounts of bone marrow (1/2, 1, 2 femurs). All cultures proved to be alike in hematopoiesis maintenance, though the MSC content in them corresponded to the

Dose of irradiation	Foci size, % of control
0	100 ± 18.8
1.5-2.5	156 ± 20
4.0-6.0	200 ± 37.5
7.0-8.0	210 ± 31.2
10.0-13.0	290 ± 68.7

Table 13: The size of the ectopic foci in irradiated recipients

Transfer number	Cells in the foci, $x10^6$
1	15.9
2	18.1
3	31.0
4	18.2
5	38.4
6	28.4
7	35.2
8	20.1

Table 14: Cellularity of ectopic bone marrow foci on repeated transplantation into irradiated recipients

Experiment №	Culture age, weeks	Non-irradiated recipients		Irradiated recipients	
		Foci formed/ number of implants	Cellularity, $x10^6$	Cellularity, $x10^6$	Cellularity, $x10^6$
1	2	2/2	0.7	2/2	22.5
2	4	0/2	--	2/2	30.5
3	4	2/2	8.0	1/2	7.6
4	4	2/2	5.7	2/2	35.5
5	5	2/2	9.3	2/2	55.0
6	6	6/6	2.9	2/2	105.0
7	9	4/4	4.8	4/4	12.5

Table 15: Size of ectopic foci produced by adherent cell layer from LTBMC in non-irradiated and irradiated recipients

dose of plated bone marrow (see Table 7). At the same time, in irradiated recipients all cultures produced foci approximately the same size (37.1, 42.7 and 38.3x10^6 correspondingly) [12].

The effect of hydroxyurea was estimated by the size of ectopic foci formed from treated ACLs in irradiated and non-irradiated recipients [20]. The results were similar in both types of recipients. So one may conclude that during the ACL formation both types of stromal precursors actively proliferate. MSCs functioning during the transfer into non-irradiated recipients and the more mature precursors taking part in the foci formation in the irradiated recipients.

The data suggest that the compartment of the hemopoietic stromal precursors is heterogenic and includes cells of at least two differentiation levels. Those less differentiated, MSCs able to transfer a hematopoietic microenvironment are marked by a relatively high self-maintenance, do not respond to the systemic demand of the irradiated recipient, and create a microenvironment, the size of which is proportional to the number of transferred MSCs. More mature precursors are marked by poor self-maintenance; they respond to the systemic demand of the irradiated recipient and, in the culture, to the surface of the flask bottom, and are not transplantable via in vivo transfer.

Summarizing the data presented above, the compartment of stromal bone marrow cells has a hierarchical structure. There are true mesenchymal stem cells (MSCs) capable of both self-maintaining and differentiating into all stromal lineages, and more mature inducible stromal precursors, which keep the ability for multipotential differentiations while losing their self-renewing ability. MSCs are mainly in mitotic rest and are not sensitive to the systemic demand, while more mature precursors proliferate more easily in the case of systemic requirements.

More mature inducible stromal precursors are sensitive to the unknown factor(s) released after irradiation. When injected during foci formation sera from irradiated mice also stimulate these precursors, resulting in increased size of the foci formed — the size of the foci was 19.5±1. 8x10^6 compared with 14±1.5x10^6 in control mice [19]. Addition of sera from irradiated mice to the LTBMC also increases the number of stromal cells in the ACLs (Table 16) [21].

In order to define the organ producing the unknown factor(s), various organs of irradiated mice were co-transplanted simultaneously with the implantation of the BM plug under the renal capsule. Intravenous injection of irradiated sera as well as implantation of 5–6 irradiated bones under the skin enhanced the growth of stromal cells in the foci formed (Table 17) [21].

Thus, the unknown factor(s) stimulating the growth of stromal inducible precursor cells are produced in the irradiated bones and secreted into the blood serum.

Group	Cell number per well, x 10^3
control (no additional serum)	118 ± 4
2% of sera from non-irradiated mice	131 ± 7
0.5% of sera from irradiated mice	160 ± 4
1 % of sera from irradiated mice	185 ± 5
2 % of sera from irradiated mice	206 ± 5

Table 16: Influence of the sera from irradiated mice on the number of cells in the ACLs from LTMBC

recipients	The organ from irradiated mice, transplantation site	Foci size, x 10^6
intact (non-irradiated)		9.0 ± 1.7
irradiated		21.1 ± 6.2
intact	BM from 6 femurs, under the skin	8.6 ± 2.3
intact	5–6 femurs, under the skin	22.0 ± 4.1
intact	Thymus, under the renal capsule	14.8 ± 3.4
intact	Equivalent of ½ of the spleen, intravenously	10.5 ± 1.2
intact	1/3 of the spleen, under the renal capsule	11.8 ± 3.3
intact	the spleen, under the skin	13.1 ± 2.8
intact	1/3 of the liver, under the skin	14.8 ± 3.2
intact	Sera from irradiated mice, intravenously	20.1 ± 1.5
intact	Sera from non-irradiated mice, intravenously	14.0 ± 2.8

Table 17: Analysis of the various organs of irradiated mice for their stroma-stimulating activity

G-CSF treatment	Bone marrow implantation			Foci retransplantation		
	Implant number	Cellularity, x10^6	Ossicle weight, mg	Implant number	Cellularity, x10^6	Ossicle weight, mg
Control	4	8.3±1.7	2.2±0.4	4	16.2±3.3	2.3±0.5
10 days	4	5.3±0.7	2.7±0.7	4	9.0±1.8	1.9±0.5
17 days	6	3.4±0.6	2.0±0.4	6	1.7±0.3	1.3±0.4

Table 18: Influence of G-CSF on the ectopic foci formation from the bone marrow of intact mice

The regulation of MSCs in vivo by hematopoietic growth factors

Regulation of MSCs by soluble factors remains obscure. During the formation of the hematopoietic microenvironment MSCs are affected by G-CSF. When recipients of BM were injected over 10 or 17 days beginning from the day after implantation of BM under the renal capsule it dramatically decreased size of the foci formed (Table 18).

Obviously, when administered during the active proliferation and differentiation of MSC, G-SCF inhibits their proliferation (judging from the decreased size of the foci formed); moreover, it diminishes the number of MSCs themselves as was shown by retransplantation of the foci [22].

Group	Bone marrow implantation			Foci retransplantation		
	Implant number	Cellularity, x10^6	Ossicle weight, mg	Implant number	Cellularity, x10^6	Ossicle weight, mg
Control	9	6.5±1.0	2.0±0.2	4	5.9±1.2	2.6±0.4
G-CSF, 6 days	4	7.4±2.2	1.1±0.2			
G-CSF, 10 days	3	10.7±2.0	1.0±0.2	3	11.3±2.9	3.0±0.6
G-CSF, 17 days	4	9.2±1.0	3.3±0.7	3	13.3±2.8	3.5±0.7
G-CSF+SCF, 6 days	4	6.4±1.6	2.2±0.5			
G-CSF+SCF, 10 days	4	12.8±1.5	2.4±0.6			
G-CSF+SCF, 17 days	4	18.4±3.9	1.7±0.5	5	19.5±4.2	3.2±0.6

Table 19: Size of the ectopic foci formed from the bone marrow of mice treated with cytocines

Conversely, cytokine treatment of the MSCs in their steady-state (in the intact bone marrow increases the number of these stromal precursors (Table 19).

The combination of G-CSF with SCF and the longest treatment (for 17 days) had a maximal effect on the MSC number in the bone marrow of treated mice. In the case of G-CSF treatment this effect was transient and did not last for a month, in the case of cytokine combination, however, the increase in the number of MSCs was stable for at least a month. Cytokine treatment did not affect the osteogenic potential of MSCs either during stroma formation or in the intact bone marrow [22].

Thus, during the building of ectopic hematopoietic foci when stromal precursors are proliferating and differentiating, pharmacological concentrations of G-CSF inhibit the process of foci formation and diminish the number of stromal precursors in them. When affecting the mature non-proliferating bone marrow stroma, cytokines increase the number of MSCs.

Conclusions

Summarizing the works of J. L. Chertkov, it is possible to describe the main features of MSCs. These cells are capable of transferring a hematopoietic microenvironment due to both their high proliferative potential and their ability to differentiate into all bone marrow stromal lineages, including bone, cartilage, and marrow stromal cells. MSCs are more radioresistant than HSCs but they still suffer from radiation and their damage could not be fully recovered. Impaired hematopoiesis also influences the MSCs. The compartment of MSCs and stromal precursor cells is organized hierarchically. Therefore, J. L. Chertkov described the main features of MSCs from many sides using the functional assay.

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Gurevich, O. A., Udalov, G. A., Samoylina N. L., Samoylova R. S., Lemeneva N. L., Todria T. V., Olovnikova N. I., Olshanskaya Y. V., Shiponova (Nifontova) I. N., Ershler M. A. and Drize N. I. as co-authors of his works.

References

1. Drize NJ, Keller JR, Chertkov JL. Local clonal analysis of the hematopoietic system shows that multiple small short-living clones maintain life-long hematopoiesis in reconstituted mice. Blood. 1996;88(8):2927-2938.

2. Drize N, Chertkov J, Sadovnikova E, Tiessen S, Zander A. Long-term maintenance of hematopoiesis in irradiated mice by retrovirally transduced peripheral blood stem cells. Blood. 1997;89(5):1811-1817.

3. Olovnikova NI, Drize NJ, Ershler MA, Nifontova IN, Belkina EV, Nikolaeva TN, Proskurina NV, Chertkov JL. Developmental fate of hematopoietic stem cells: the study of individual hematopoietic clones at the level of antigen-responsive B lymphocytes. Hematol J. 2003;4(2):146-150.

4. Drize NJ, Olshanskaya YV, Gerasimova LP, Manakova TE, Samoylina NL, Todria TV, Chertkov JL. Lifelong hematopoiesis in both reconstituted and sublethally irradiated mice is provided by multiple sequentially recruited stem cells. Exp Hematol. 2001;29(6):786-794.

5. Chertkov JL, Gurevitch OA. Radiosensitivity of progenitor cells of the hematopoietic microenvironment. Radiat Res. 1979;79(1):177-186.

6. Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991;9(5):641-650.

7. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy. 2005;7(5):393-395.

8. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells in vitro. J Cell Physiol. 1977;91(3):335-344.

9. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation. 1968;6(2):230-247.

10. Udalov GA, Gurevich OA, Chertkov IL. Origin of hematopoietic cells in a syngenous and semisyngenous focus of heterotopic hematopoiesis. Biull Eksp Biol Med. 1977;83(5):584-586.

11. Chertkov JL, Drize NJ, Gurevitch OA, Udalov GA. Hemopoietic stromal precursors in long-term culture of bone marrow: I. Precursor characteristics, kinetics in culture, and dependence on quality of donor hemopoietic cells in chimeras. Exp Hematol. 1983;11(3):231-242.

12. Chertkov JL, Drize NJ, Gurevich OA. Hemopoietic microenvironment and hemopoietic stroma precursors. Recent advances in haematology immunology and blood transfusion : proceedings of the plenary sessions of the joint meeting of the 19th Congress of the International Society of Haematology and the 17th Congress of the International Society of Blood Transfusion, Budapest, August 1-7, 1982, Eds. Susan R. Hollán; International Society of Haematology, 12,1983;133-147.

13. Gurevich OA, Drize NI, Chertkov IL. Importance of cell contacts for the differentiation of the precursor cells of hematopoietic stroma in long-term bone marrow cultures. Biull Eksp Biol Med. 1982;94(8):97-100.

14. Chertkov JL, Drize NJ, Gurevitch OA, Samoylova RS. Origin of hemopoietic stromal progenitor cells in chimeras. Exp Hematol. 1985;13(11):1217-1222.

15. Chertkov JL, Gurevitch OA. Self-maintenance ability and kinetics of haemopoietic stroma precursors. Cell Tissue Kinet. 1980;13(5):535-541.

16. Chertkov JL, Gurevich OA, Udalov GA. Role of bone marrow stroma in the phenomenon of hybrid resistance. Biull Eksp Biol Med 1979 Apr; 87(4):337-40.

17. Gurevich OA, Chertkov JL. Radiosensitivity of hemopoietic stroma precursors from long-term bone marrow culture. Radiobiology. 1983;23(4):521-523.

18. Gurevich OA, Drize NI, Udalov GA, Chertkov IL. Effect of hematopoiesis on progenitor bone marrow stromal cells. Biull Eksp Biol Med. 1982;94(10):115-117.

19. Chertkov JL, Gurevitch OA. Hematopoietic stem cell and its microenvironment. Moscow, Meditzina 1984. Russian.

20. Gurevich OA, Drize NJ, Chertkov JL. Proliferation of cells-precursors of hematopoietic microenvironment in murine long-term bone marrow culture. Bull Exp Biol Med. 1984;XCVIII(11):612-614.

21. Drize NI, Ershler MA, Chertkov IL. Radiation-induced hemopoietic cell growth factor: detection in a culture. Bull Exp Biol Med. 2001;132(6):1213-1215.

22. Drize NJ, Chertkov JL. Influence of cytokine (G-CSF and SCF) treatment on the murine precursors of hematopoietic stroma. Bull Exp Biol Med. 1998;125(2):204-206.

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Клеточная терапия и трансплантация (КТТ), том 2, номер 6

Клетки-предшественницы кроветворного стромального микроокружения

Иосиф Л. Чертков (†), Ольга А. Гуревич, Геннадий А. Удалов (†),
Ирина Н. Шипунова, Нина И. Дризе

Резюме

Данная работа суммирует достижения профессора Иосифа Львовича Черткова в изучении клеток-предшественниц кроветворного стромального микроокружения. В своих работах Чертков использовал уникальный функциональный метод анализа стромальных клеток-предшественниц – метод образования очагов эктопического кроветворения под капсулой почки сингенных с донорами костного мозга реципиентов. Было продемонстрировано наличие в костном мозге мезенхимальных стволовых клеток, способных к переносу кроветворного микрооружения, т.е. дифференцировке во все стромальные клеточные линии, и к самоподдержанию, т.е. многократному переносу кроветворного микроокружения. Были изучены радиочувствительность и пролиферативный потенциал мезенхимальных стволовых клеток. Показана важность сохранения межклеточных контактов для построения стромального микроокружения in vitro и in vivo. Выявлена иерархичная структура отдела мезенхимальных стволовых клеток и охарактеризован отдел более дифференцированных, индуцибельных клеток-предшественниц стромального микроокружения. Показано взаимное влияние кроветворных и стромальных клеток. Охарактеризованы некоторые пути регуляции стромальных предшественников. Данная компиляция работ И.Л.Черткова демонстрирует его вклад в изучение регулирующего кроветворение стромального микроокружения костного мозга.

Ключевые слова: стромальное микроокружение, мезенхимальные стволовые клетки, индуцибельные стромальные клетки-предшественницы, очаг эктопического кроветворения, длительная культура костного мозга