

Regeneration and Carcinogenesis

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Abstract

Introduction: Once people reach 40 years of age, they have a decrease in their pool of pluripotent stem cells, and an increased risk for development of oncological diseases.

Materials and methods: The first part of the study was conducted in 11 patients aging 54 to 76 years old with cancer of the kidney, bladder, or prostate in stages III-IV of the disease. The second part of the study was conducted in four patients aged 60-82 years old, who were given from 4 to 7 transfusions of mononuclear fraction of peripheral blood from young donors 19-23 years old, with the same sex and blood types as the recipients, in order to restore cell regeneration.

Results: In the first part of the study, 1 month after chemotherapy or targeted therapy, all 11 cancer patients had leukopenia accompanied by an increase in the contents of FGFb in the blood by 1.74 times on average. Four of these patients had an increase in the level of human VEGF-A of 1.25 times on average, while three patients had an increase in the level of human EGF of 1.13 times on average. In the second part of the study, 3-6 months after the completion of a cycle of 4-7 blood transfusions of mononuclear fraction of periphery blood, four patients had an increase in the contents of hematopoietic progenitor cells CD34+ of periphery blood by 3.25 times on average, to the level normal in young people, while the level of FGFb decreased by 1.78 times on average. Among two patients, the level of human VEGF-A decreased by 1.48 times on average, while for three patients the level of human EGF decreased by 4.12 times on average. In the buccal epithelium, all four patients had a decrease in the expression of p53 by 6.02 times on average, while three of them had a decrease in the expression of Bcl-2 by 60.0 times on average.

Conclusion: Violation of tissue renewal is a major cause of carcinogenesis in people older than 40 years old. Excessive stimulation of mitotic activity among people over 40 can be reduced to normal levels by restoring the pool of pluripotent stem cells through transfusion of mononuclear fraction of peripheral blood from young donors 18-23 years old with the same blood groups and sex as the recipient (RF patent number 2350340).

Keywords: regeneration, carcinogenesis, pluripotent stem cells, testosterone, AR, FGFb, Bcl-2, p53

Introduction

Once people reach 40 years of age, they have a decrease in their pool of pluripotent stem cells [1], leading to atrophy and fibrosis in all tissues and organs, as well as an increased risk of cancer [2, 3, 4]. A study of the link between failed tissue renovation and carcinogenesis is of considerable interest.

Objective

Study the causes and ways to normalize increased mitogenic stimulation in people over 40 years old.

Materials and methods

Studying the dependency of mitogenic stimulation on changes in tissue renewal (regeneration) in people over 40 years old was carried out using the example of intensification of existing violations in regeneration among cancer patients receiving chemotherapy/targeted therapy, and using the opposite example of the recovery of regeneration in patients older than 50 years old who were provided with a transfusion of mononuclear fraction of peripheral blood from young donors 19-23 years old. In both the first and second cases, changes in mitogenic stimulation occurred over a relatively short period of time, which suggested they were connected to worsening regeneration disorders, or, alternatively, to restoration of regeneration. In the first case, mitogenic factors – cell growth factors were determined in 11 patients aged 54 to 76 years old with cancer of the kidney, bladder, or prostate cancer in the III – IV stages of the disease before and 1 month after the initiation of chemotherapy or targeted therapy. Since pluripotent stem cells are involved in the renewal of all body tissues [4], when there is suppression of the pool of stem cells during chemotherapy/targeted therapy (as confirmed by the development of leukopenia), all patients showed a violation of tissue renewal (regeneration disorder). The criteria for inclusion

in the main group were the following: undergoing chemotherapy or targeted therapy for various cancers, and the patients' age, which was over 50 years old. The control group consisted of 11 apparently healthy blood donors between the ages of 18 to 23 years old. Selection criteria were the following: apparently healthy people aged 18 to 25 years old.

In the second case, mitogenic factors – cell growth factors were detected in four patients aged 60 to 82 years old, who received from four to seven transfusions of mononuclear fraction of peripheral blood from young donors 19 to 23 years old at intervals of 2 to 3 months, with the aim of restoring the tissue renewal process (restoration of regeneration).

The criteria for inclusion in the main group were the following: patient age older than 50 years old and transfusion to these patients of allogeneic mononuclear fraction peripheral blood in order to restore regeneration. Procurement of mononuclear fraction peripheral blood for each of the four recipients was made from one young donor 19-23 years old. The donor selection criteria were the following: compliance to the health requirements of the Order of the Ministry of Health of the Russian Federation dated September 14, 2001 № 364 "On approval of the medical examination of donor blood and its components," an age of 18 - 23 years old, the same gender as the recipients, and the same antigen systems ABO, Rh-factor, phenotype Rh-factor, and Kell (Russian Federation patent 2350340) as the recipients. For Kell (+), transfusion was carried out from a donor with Kell (-). The control group consisted of four healthy blood donors between the ages of 19 to 22 years old. The selection criteria were the following: apparently healthy people aged 18 to 25 years old.

Evaluating the effectiveness of treatment aimed at restoring regeneration was carried out based on determining the number of hematopoietic progenitor cells CD34+ of peripheral blood. Additionally, after obtaining patients' consent, sampling of biopsies of oral mucosa was performed under local anesthesia for conducting histological and immunohistochemical studies. The choice of the buccal epithelium was random, and was conditioned by the simplicity of sampling this material. Changes in the buccal epithelium were interpreted as a reflection of overall changes in the tissues of patients receiving transfusions of mononuclear fraction of peripheral blood.

Procurement of donors' mononuclear fraction of peripheral blood was carried out using an Amicus separator and an Amicus MNC-Kit with a specific one-use sterile expenditure system. During the first transfusion, in order to form a chimerism to minimize the antigen load, there was a reduced intake of mononuclear fraction of peripheral blood: 5 cycles of separation (the approximate volume of intraoperative treated blood of the donor—1,500 ml). For subsequent transfusions, carried out in order to restore tissue renewal (regeneration restoration), mononuclear fraction of peripheral blood was obtained by carrying out 15 separation cycles with processing of $4,600 \pm 100$ ml of donor blood. Settling of erythrocytes was performed using 20 ml of Stabizol hemocorrector solution. Donors underwent a standard checkup before procurement of mononuclear fraction of peripheral blood was conducted. Donors were prepared for five days before the leucopheresis operation. Donors, who

were men with a body weight of 75 kg on average, were injected subcutaneously with filgrastim (brand name – Neipomax®) in a dose of 78 mln units/30 + 48 mln units (780 µg/300 + 480 µg) once per day, daily, over the course of 5 days. For women, who had a lower body weight than men (60 kg on average), the dose of filgrastim (Neipomax®) was 60 mln units/30 + 30 mln units (600 µg/300 + 300 µg) once per day, daily, over the course of 5 days. The last filgrastim administration was done on the day of separation. At the end of the transfusion of mononuclear fraction of peripheral blood, patients were administered one dose of 50 mg of Prednisolone intravenously to reduce the severity of post- transfusion reactions. Starting from the first hours after transfusion to patients – men with a body weight of 80 kg on average – filgrastim (Neipomax®) was subcutaneously injected at a dose of 78 mln units/30 + 48 mln units (780 µg/300 + 480 µg) once per day, daily, over the course of 10 days. For women, who had a lower body weight than men (60 kg on average), the dose of filgrastim (Neipomax®) was 60 mln units/30 + 30 mln units (600 µg/300 + 300 µg) once per day, daily, over the course of 10 days. Injecting colony-stimulating factor (filgrastim) was done for transfused mononuclear leukocytes to overcome the restriction point as they enter the alien blood environment of the recipient, as well as for subsequent stimulation of their division and an increase in their numbers. Additionally, for the same purpose, patients were administered Methyluracil tablets 0.5 g, 1 tablet, four times a day, for 1 month.

Research Methods

Measuring peripheral blood hematopoietic CD34+ progenitor cells was performed by flow cytometry on a FC500 flow cytometer using a set of Stem-Kit Reagents made by Beckman Coulter Company (France). The sensitivity of the method for determining the concentration of peripheral blood hematopoietic CD34+ progenitor cells was 0.5 cells/mcl. The coefficient of variation was 10.7%.

Enzyme immunoassay examination. Measuring human fibroblast growth factor with a basic shape (FGFb) in the blood serum was carried out using a test set made by the company R & D Systems, Inc., with a test-sensitivity of 3.0 pg/ml, and a coefficient of variation of 5.3%. Measuring human vascular endothelial growth factor A (VEGF-A) was carried out using a test set made by Bender MedSystems, with a test-sensitivity of 7.9 pg/ml, and a coefficient of variation of 6.2%. Measuring human epidermal growth factor (human EGF) was carried out using a test set made by Bender MedSystems, with a test- sensitivity of 0.26 pg/ml, and a coefficient of variation of 3.5%.

Morphological examination. Pieces of the oral mucosa were fixed in a 10% solution of neutral formalin, were put through alcohols, and were embedded in paraffin according to standard methods of histological preparations [5]. Sections 5 µm thick were stained with hematoxylin and eosin (H&E section).

Immunohistochemical studies. Measuring the expression of androgen receptors (AR), as well as the expression of p53 and Bcl-2 was carried out using a one-step method for unmasking the antigen (using a method of high-temperature tissue treatment) on paraffin sections using a diagnostic kit produced by Novocastra Laboratories Ltd (Great Britain) for AR and p53, and by Dako (Denmark) for Bcl-

2. AR identification results were assessed using a semiquantitative histochemical score method [6]. The values of p53 and Bcl-2 are presented as a percentage of the number of positive cells to 1000 cells scanned.

Statistical analysis.

Statistical analysis was performed using the methods of variance analysis of repeated measures and variance analysis for comparing two groups using Student's t-test. All data in the text and tables are presented as average values and standard deviations ($M \pm \sigma$). Furthermore, Student's t-test (t) values are presented [7].

Results and Discussion

In the first part of the study, 1 month after chemotherapy or targeted therapy, all 11 cancer patients developed leukopenia, accompanied by an increase in basic fibroblast growth factor (FGFb) in the blood by an average of 1.74 times. That said the original average level of basic fibroblast growth factor (FGFb) before treatment in all 11 cancer patients was

5.5 times higher than the analogous level among young people in the control group (Tables 1, 2). Also, among cancer patients of the main group, following chemotherapy/targeted therapy there was a tendency towards an increase in the levels of human vascular endothelial growth factor A (VEGF-A) and human epidermal growth factor (human EGF). Thus, of the 11 cancer patients, 4 of them had an increase in the level of human vascular endothelial growth factor A (VEGF-A) by 1.25 times on average (from $351.1 \pm$

189.7 pg/ml to $439.4 \pm 161,7$ pg/ml), while 3 patients had an increase in the level of human epidermal growth factor (human EGF) by 1.13 times on average (from $217.8 \pm 22,9$ pg/ml to $246.4 \pm 2,5$ pg/ml) (Tables 3, 4).

In the second part of the study, 3 to 6 months after completing the cycle of 4 to 7 transfusions of mononuclear fraction of peripheral blood, 4 patients showed an increase in hematopoietic progenitor cells CD34+ of peripheral blood by 3.3 times on average (from 1 to 2 - 5 cells in 1 mcl, to 3.3 cells per 1 mcl on average). A comparison of the result received with the instructions of Stem-Kit Reagents by Beckman Coulter (France) on 117 healthy individuals of various age groups with the maximum level of CD34+ of peripheral blood among young people – 6.5 Cells/mcl, and the minimal level among older individuals – 0.5 Cells/mcl, showed that among patients who received the cycle of transfusions of mononuclear fraction of peripheral blood from donors 19 – 23 years old, the level of hematopoietic progenitor cells CD34+ of peripheral blood increased to a level characteristic of young men. This indicates that it was possible among 60 - 82 year old patients to restore the stem cell pool to the level of young individuals (Table 5, 8). After restoration of the pool of stem cells among 4 patients from 60 - 82 years old, and therefore of the regeneration process, to the level among young people, 3 to 6 months after completing the cycle of transfusions of mononuclear fraction of peripheral blood there was a decrease in the level of basic fibroblast growth factor (FGFb) by 1.78 times on average (Table 5). The average amount of basic fibroblast growth factor (FGFb) in the blood serum of patients who received transfusions of mononuclear fraction of peripheral blood exceeded the average value of this indicator in the group of young healthy blood donors prior to transfusion by 2.7 times (Table

6). After completion of the cycle of transfusions of mononuclear fraction of peripheral blood, a comparison of the average values of basic fibroblast growth factor (FGFb) among patients of the main and control groups was not statistically significant (Table 7). Furthermore, among patients receiving transfusions of mononuclear fraction of peripheral blood, following completion of the cycle of 4–7 transfusions, there was a tendency towards a decrease in the levels of human vascular endothelial growth factor A (VEGF-A) and human epidermal growth factor (human EGF). Of them, in 2 patients the level of human vascular endothelial growth factor A (VEGF-A) decreased by 1.48 times on average (from $209.1 \pm 4,5$ pg/ml to $140.8 \pm 48,6$ pg/ml), and in 3 patients the level of human epidermal growth factor (human EGF) decreased by 4.12 times on average (from 146.6 ± 84.3 pg/ml to 35.5 ± 15.1 pg/ml)

(Table 9). A reduction in the levels of cell growth factors naturally resulted in all 4 patients to a reduced expression of p53 by 6.02 times on average in the buccal epithelium, and in 3 of them to a reduced expression of Bcl-2 by 60.0 times on average. Restoration of the number of Leydig cells and the patient's own testosterone production in patients over

40 years old, observed during transfusion of mononuclear fraction of peripheral blood from young donors 18-23 years old [8], resulted, among patients of the main group of males (3 patients), in a reduced expression of androgen receptors (AR) by 2.12 times on average (Table 5). All four patients 60 – 82 years old showed a thickening of the epithelial layer due to a more pronounced development of the basal and parabasal layers, an increase in the number of small blood vessels, a disappearance of parakeratosis phenomena, a disappearance or a reduction of dystrophic-modified epithelial cells (cells with optical empty cytoplasm), and an increase of lymphocytic infiltration after completion of transfusion of mononuclear fraction of peripheral blood from donors 19–23 years old, upon histological examination of the epithelium of human buccal mucosa.

Among vertebrate populations with differentiated cells subject to renewal, there is continuous destruction of old cells and their replacement by new cells. The cell renewal can occur by simple division to form two daughter cells of the same type, or through precursor cells of cambial zones. Cambial cells form progeny during division, some of which continue to differentiate, while other progeny remain poorly differentiated [9]. Committed progenitors and differentiated cells, having embarked on the path of differentiation or having completed it, can divide a limited number of times [9] and are unable to ensure tissue regeneration throughout the entire ontogeny [4]. Tissue renewal over such a long time period is impossible without the participation of a specialized system responsible for regeneration. A part of this system are pluripotent stem cells that can migrate and differentiate into all types of somatic cells and into a line of germ cells, as well as have the ability to self-renew throughout the life of the organism [4]. The basal membrane, underlying the epithelium layer, does not prevent migration of stem cells to replenish the pool of poorly differentiated cells of the cambial zone, ensuring the replacement of old cells [9]. Pluripotent stem cells are a separate branch of the differentiation of embryonic cells [9], providing for regeneration of all body tissues of the organism throughout ontogeny [4]. Old cells feature desialation of the cell surface and infringement of protection of terminal membrane glycoproteins containing mannose. The emergence of free mannose on the surface of cells makes them available for recognition by

macrophages. The first line of immune defense – inflammation – begins as a reaction of natural immunity based on a phylogenetically more ancient defensive process. During necrosis (apoptosis) of old cells, and the simultaneous macrophage inflammation, as well as the surrounding epithelial and endothelial cells, the stromal cells of hematopoietic and lymphoid organs form colony-stimulating and cell growth factors, interleukins [10, 11]. Colony-stimulating factors induce proliferation of pluripotent stem cells [9] for their subsequent admission to cambial areas or directly to the place of cell death of old cells [4]. Cell growth factors, acting in various combinations, selectively stimulate the proliferation and differentiation of progenitor cells of cambial zones [9]. Management of the processes of cell differentiation is carried out by a corresponding part of the program of development, initiating the local formation of cell growth factors by the cellular environment in a strict sequence, as well as the emergence of complementary receptors in precursor cells. Incretion of cell growth factors continues until the full restoration of damaged tissue or the formation of fibrous tissue at the injury site by fibroblasts activated by the growth factors. When there are sufficient numbers in the pool of pluripotent stem cells, such as in young people, the replenishment by pluripotent stem cells of cellular composition of cambial zones and the subsequent regeneration of tissues adequately counters the death of old cells. After the replacement of old dead cells with new cells, local production of cell growth factors and colony-stimulating factors is terminated. Short-term physiological formation of cell growth factors does not lead to malignant transformation of tissues [4].

Management of stem cell migration is conducted through the formation in several stages of tissue-specific receptors. Initially, in response to desialation and the appearance on the surface of old or intensively proliferating cells of glycoproteins with free end mannose, there is binding of antigen-presenting cells (macrophages and other) with tissue specific antigens. Antigen-presenting cells deliver tissue-specific antigens of old dead cells through lymph nodes or other lymphoid organs to T-helpers [4, 11].

Table 1. The average content of basic fibroblast growth factor (FGFb) in the blood of cancer patients before chemotherapy/targeted therapy and in the group of young, apparently healthy blood donors

Research groups \ Indicators	FGFb, pg/ml
The main group of patients with cancer of the prostate, bladder and kidney in stage III - IV of the disease before chemotherapy/targeted therapy (n = 11)	5.0 ± 2.6
Control group of young, apparently healthy blood donors (n = 11)	0.9 ± 0.8
t	4.917
p	p < 0.001

Note: FGFb - basic fibroblast growth factor.

The examined factors, which develop during the death of old cells and the inflammation that develops concurrently, lead to the initiation of malignant transformation of cells and determine the mechanisms of progression of tumor growth. [4] On the periphery of the tumor is a zone of perifocal (demarcation) inflammation. Among other cells in this zone are macrophages [10, 18], which are drawn by free mannose of the desialation surface of intensively proliferating cells and other antigens produced during necrosis/apoptosis of cells [4]. Like osteoclasts (generated from monocytes and being a variety macrophages) that

destroy bone matrix during bone turnover [9], macrophages of the zone of perifocal inflammation, releasing hydrolytic enzymes, lyse the surrounding tissues (destroy endothelium, basement membranes, fibronectin, collagen, elastin, bone matrix and other structures) to make room for tumor cells. Intense formation of cell growth factors that stimulate the proliferation of endothelial cells (vascular growth factor and others secreted by macrophages), results in the proliferation of endothelial cells and the formation of new blood vessels (angiogenesis). In turn, the endothelium, under inflammatory conditions, produces basic fibroblast growth factor and platelet-derived growth factor, which enhance proliferation, and also forms IL-7, which causes the expression of Bcl-2. The expression of Bcl-2, increasing resistance to cell death through the apoptosis mechanism [11], screens the endothelium, migrating stem cells, and newly formed malignant cells from the resulting highly cytotoxic products of cells and macrophages [4]. Confirmation that the main reason for the increase of mitogenic stimulation and the increase in the formation of cell growth factors in people over 35- 40 years old is the incompleteness of tissue renewal (incompleteness of regeneration), caused by the reduction in the pool of stem cells, comes from the increased production of basic fibroblast growth factor (FGFb) 1 month after the start of chemotherapy or targeted therapy for all cancer patients observed. On the background of developing leukopenia, indicating the suppression of the pool of stem cells by chemotherapy drugs/targeted therapy and worsening disorders of tissue renewal (violation of regeneration) in all 11 patients of the main group of the first phase of the study, who had initially elevated levels of basic fibroblast growth factor (FGFb) in the blood, a further increase in formation of this factor occurred in the blood by an average of 1.74 times (Table 2). Furthermore, among the main group of cancer patients, after chemotherapy/targeted therapy there was a tendency towards an increase of levels of human vascular endothelial growth factor (VEGF-A) and human epidermal growth factor (human EGF) in the blood. In 4 of these patients the increase in the level of human vascular endothelial growth factor (VEGF-A) averaged 1.25 times, and in 3 of them there was an increase in human epidermal growth factor (human EGF) of 1.13 times on average (Tables 3-4).

Thus, the cytostatic effect not only on the tumor, but also on the pluripotent stem cells of the bone marrow, which takes place when conducting chemotherapy or targeted therapy, leads to more stimulation of mitogenic activity, and an increased risk of tumor recurrence and the risk of its metastasis, as well as to the appearance of new tumors in other localities. Pathological processes similar to those described above are typical of patients with rheumatological diseases, treated with immune suppressive therapy, as well as for all people over 35- 40 years old, who have a reduction in their pool of pluripotent stem cells and have a consequent disorder of tissue renewal. Both groups have an increased risk of malignant diseases.

Table 2. Average content of basic fibroblast growth factor (FGFb) in the blood in cancer patients before and one month after the start of chemotherapy/targeted therapy

Research groups/Indicators	FGFb, pg/ml
The main group of patients with cancer of the prostate, bladder and kidney in stage III-IV of the disease before chemotherapy/targeted therapy (n = 11)	5.0 ± 2.6
The main group of patients with cancer of the prostate, bladder and kidney in stage III - IV of the disease one month after the start of chemotherapy/targeted therapy (n = 11)	8.7 ± 4.7
t	3.607
p	p < 0.005

Note: FGFb – basic fibroblast growth factor.

On the surface of the activated endothelium, platelets, and white blood

cells, one can observe expression and adhesion of coagulation factors leading to fibrin formation [9, 18]. Adaptive changes in the endothelium in the area of inflammation are accompanied by intra- and extravascular clotting of fibrinogen and the formation of blood clots. Necrosis develops as a result of thrombosis of the blood vessels around the inflamed tissue area. Accordingly, tumors are often subject to necrosis and ulceration [10]. Due to incompleteness of the regeneration process following the death of old cells, and the presence of accompanying local inflammation sites, there is a greater risk of blood clots as well in the majority of tissues in people older than 35-40 years of age [4].

During mitosis, the cells are rounded and lose their strong bond with each other (due to a reduction in cell adhesion and loss of cell-cell contacts). The integrity of the tissue consisting of such cells is disrupted [9]. For this reason, metastasis begins faster in tumors with the highest intensity of division of cancer cells [4]. Malignant cells, the formation of which is directed at fulfilling a deficit of pluripotent stem cells, acquire a similarity to these cells (e.g., embryonic antigens appear on these cells). During metastasis, malignant cells repeated the path and mechanisms of migration of stem cells in tissue regeneration. By analogy to the pluripotent stem cells, in order for the malignant cells to have contact with T-helper cells, and for the formation in them of tissue-specific receptors, these cells must be continuously circulated through the secondary lymphoid organs. Tumor cells attach to endothelial cells of postcapillary venules, squeeze between them, and flow through the lymphatic vessels to the lymph nodes and then through the corresponding groups of lymph nodes and blood vessels into the thoracic duct, through which they return to the blood. This circulation occurs constantly, leading to tumor dissemination [4]. The focus of metastasis of malignant cells is determined by the formation of the corresponding tissue-specific receptors on their surface. This is preceded by binding in secondary lymphoid organs of antigen presenting cells (carrying complexes of tissue specific antigens of dead old cells on their surface) with T-helper cells, activation of T-helper cells, and the subsequent cooperation (mediated by antigen-presenting cells) of T-helper cells with tumor cells to form tissue-specific receptors on their surface (old dead cells complementary to tissue specific antigens) [4]. The appearance of tissue-specific "homing receptors" determines the direction of migration of malignant cells to the places of death of old cells. The predominance of the death of old cells over the regeneration process in most tissues in people older than 35-40 years old, accompanied by the appearance of an excess of tissue-specific chemoattractant (which tissue-specific antigens of dead old cells serve as), promotes, together with a reduction in adhesion and the loss of contacts between intensively proliferating cells, metastasis of malignant tumors [4]. After contact with T-helper cells and the formation of tissue-specific receptors, cancerous cells, entering the places of death of old cells under the influence of the cellular environment, determining the direction of differentiation, change their histological structure, acquiring properties of progenitor cells of the cambial zone of the given tissues. This can create the illusion of development of multiple primary tumors. If metastases are formed only thanks to adhesive- cellular interactions, then proliferation of tumor cells will occur with a minimal influence of the cellular environment, without the tumor cells' inclusion in the mechanisms of local differentiation. The histological structure of such a tumor will largely correspond to the primary tumor.

Blocking osteoclasts (macrophages), as antigen presenting cells, under the use of bisphosphonates, disrupts the process of presenting antigens of dead old cells of the bone tissue to helper T-cells for formation of the corresponding receptors in tissue-specific stem cells and tumor cells. Accordingly, the effect of bisphosphonates is achieved by blocking the mechanism of migration of stem and tumor cells during regeneration of old dead cells of bone tissue. Violation of the natural mechanism of regenerating bone tissue with lysis by osteoclasts of dead old cells when

using bisphosphonates is accompanied by sequesters, consisting of non-phagocytosed conglomerates of old dead osteocytes [4].

The above-described regularities are demonstrated by the clinical example of increased levels of basic fibroblast growth factor (FGFb), human vascular endothelial growth factor A (human VEGF-A), and human epidermal growth factor (human EGF) in the blood serum of a 57 year-old patient before and 1 month after the start of targeted therapy given to the patient for kidney cancer T2N1M1 (Table 3). Similar changes were observed in a 54 year-old patient before and 1 month after the start of palliative chemotherapy for bladder cancer T2N1M1 (Table 4). These examples show an increase in production of cell growth factors and an increase through them of mitogenic stimulation in response to suppression of the pool of stem cells and the consequent violation of tissue renewal (violation of regeneration). The results show that together with their effect on the tumor, chemotherapy or targeted therapy, suppressing the pool of pluripotent stem cells and disrupting tissue regeneration, lead to the stimulation of mitogenic activity, increasing the risk of a recurrence of the tumor, its metastasis and the development of new tumors in other localizations. For this reason, during a place-localized tumor process, in order to minimize toxic effects on bone marrow the pluripotent stem cells contained in this marrow, it is advisable to conduct chemotherapy/targeted therapy in a specific region with intra-arterial selective drug injections.

To normalize the process of tissue renewal, for full replacement of old cells by progenitor cells, and to reverse the pathological processes associated with violation of regeneration individuals older than 40 years old require restoration of the numerical strength of the pool of pluripotent stem cells [4]. Positive clinical dynamics in cancer patients who have been prescribed with colony stimulating factors after chemotherapy indirectly confirms this conclusion [19]. Unfortunately, the effect of stimulation using medications with colony-stimulating factors is temporary.

Table 3. Clinical example of an increase in the levels of cell growth factors in a 57 year-old patient receiving target therapy for kidney cancer T2N1M1

Indicators\Patient	57 year-old patient, with a diagnosis of kidney cancer T ₂ N ₁ M ₁ , receiving target therapy	
	Before the start of target therapy	One month after the start of target therapy
FGFb, pg/ml	5.4	17.1
human VEGF-A, pg/ml	186.4	232.4
human EGF, pg/ml	188.1	244.6

Note: FGFb - basic fibroblast growth factor, human VEGF-A - human vascular endothelial growth factor A, human EGF - human epidermal growth factor.

Table 4. Clinical example of an increase in the levels of cell growth factors in a 54 year-old patient receiving palliative chemotherapy for bladder cancer T2N1M1

Indicators\Patient	54 year-old patient, with a diagnosis of bladder cancer T ₂ N ₁ M ₁ , receiving palliative chemotherapy (PCT)	
	Before the start of PCT	One month after the start of PCT
FGFb, pg/ml	7.9	15.2
human VEGF-A, pg/ml	655.6	686.4
human EGF, pg/ml	243.8	250.0

Note: PCT - palliative chemotherapy, FGFb - basic fibroblast growth factor, human VEGF-A - human vascular endothelial growth factor A, human EGF - human epidermal growth factor.

Conclusion

A reduction in the pool of pluripotent stem cells and the consequent violation of tissue renewal in people older than 35-40 years old are the main reasons for an increase in mitogenic activity, which leads to an increased risk of carcinogenesis. Transfusion of mononuclear fraction of peripheral blood procured from young donors 18-23 years old with the same blood groups and sex as the recipient (RF patent number 2350340), allows people over 40 years old to reestablish the pool of pluripotent stem cells and the process of tissue renewal, reduce mitogenic stimulation and the risk of cancer, improve the results of cancer treatment (aimed at removing or suppressing the growth of cancer cells), and can also be seen as a promising way to reduce biological age, while providing a significant prolongation of life and the ability to work (while maintaining a high quality of life).

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