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THE RELATIONSHIP BETWEEN AMINO ACID COMPOSITION OF BONE MARROW STROMAL FIBROBLASTS AND MYELOGRAM DATA IN DIFFERENT PHASES OF CHILDREN ACUTE LYMPHOBLASTIC LEUKEMIA

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Summary. Aim. *Determining the influence of structure and content of amino acids in stromal bone marrow fibroblasts grown in vitro, the state of hemoiesis in children with ALL*

Materials and methods. *The study involved 46 patients with ALL (I acute period, period of chemotherapy, remission) and 14 children without hematologic diseases. Studied the efficiency of cloning fibroblast colony-forming units of bone marrow, their amino acid composition, elements of myelogram.*

Results. *It is shown that in patients with ALL during all periods of the disease efficiency of cloning fibroblast colony-forming units of bone marrow was lower as compared with the healthy children. Amino acid composition in stromal fibroblasts of bone marrow had total spectrum of amino acids, except for hydroxyproline, which indicates their ability to form collagen. There was a direct correlation (rs =+0,84) between the content of proline in stromal fibroblasts and the number of erythroid progenitor cells with the myelogram of patients with ALL in remission phase.*

Conclusions. *In children with ALL stromal fibroblasts are the source of the factors necessary for the functioning for hemopoieses, including of erythroid elements, starting with the development of progenitor cells erythron.*

Key words: *children, acute lymphoblastic leukemia, stromal fibroblasts of bone marrow, amino acids, erythroid progenitor cells.*

ЗВ'ЯЗОК МІЖ АМІНОКИСЛОТНИМ СКЛАДОМ СТРОМАЛЬНИХ ФІБРОБЛАСТІВ КІСТКОВОГО МОЗКУ І ПОКАЗНИКАМИ МІЄЛОГРАМИ У ДІТЕЙ В РІЗНІ ФАЗИ ГОСТРОЇ ЛІМФОБЛАСТНОЇ ЛЕЙКЕМІЇ

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Мета роботи. *Визначення впливу складу та вмісту амінокислот в стромальних фібробластах кісткового мозку, вирощених in vitro, на стан гемопоезу у дітей з ГЛЛ (гостра лімфо-бластна лейкемія).*

Матеріали і методи досліджень. *Обстежено 46 хворих на ГЛЛ (в І гострий період, фазу хіміотерапії, ремісію) та 14 осіб без гематологічної патології. Вивчали ефективність клонування колонієутворюючих одиниць стромальних фібробластів кісткового мозку, їх амінокислотний склад, показники мієлограми.*

Результати та їх обговорення. *Показано, що у хворих на ГЛЛ в усі періоди захворювання ефективність колонієутворюючих одиниць стромальних фібробластів кісткового мозку була нижчою порівняно зі здоровими дітьми. Амінокислотний склад стромальних фібробластів кісткового мозку мав весь спектр амінокислот за вийнятком оксипроліну, що вказує на їх здатність формувати колаген. Встановлено прямий кореляційний зв'язок (r_s=+0,84) між вмістом проліну в стромальних фібробластах та числом еритроїдних клітин-попередників за даними мієлограми у хворих на ГЛЛ в фазу ремісії.*

Висновки. *У дітей з ГЛЛ стромальні фібробласти є джерелом факторів, необхідних для функціонування гемопоезу, зокрема еритроїдних елементів,* починаючи з розвитку клітин-попередників еритрону.

Ключові слова: *діти, гострі лімфобластні лейкемії, стромальні фібробласти кісткового мозку, амінокислоти, еритроїдні клітини-попередники.*

СВЯЗЬ МЕЖДУ АМИНОКИСЛОТНЫМ СОСТАВОМ СТРОМАЛЬНЫХ ФИБРОБЛАСТОВ КОСТНОГО МОЗГА И ПОКАЗАТЕЛЯМИ МИЕЛОГРАММЫ У ДЕТЕЙ В РАЗНЫЕ ФАЗЫ ОСТРОЙ ЛИМФОБЛАСТНОЙ ЛЕЙКЕМИИ

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Цель работы. *Определение влияния состава и содержания аминокислот в стромальных фибробластах костного мозга, выращенных in vitro, на состояние гемопоэза у детей с ОЛЛ (острая лимфобластная лейкемия).*

Материалы и методы. *Обследовано 46 больных ОЛЛ (в I острый период, фазу химиотерапии, ремиссию) и 14 лиц без гематологической патологии. Изучали эффективность клонирования колониеобразующих единиц стромальных фибробластов костного мозга, их аминокислотный состав, показатели миелограммы.*

Результаты. *Показано, что у больных ОЛЛ во все периоды заболевания эффективность колониеобразующих единиц стромальных фибробластов костного мозга была ниже по сравнению со здоровыми детьми. Аминокислотный состав стромальных фибробластов костного мозга имел весь спектр аминокислот, за исключением оксипролина, что указывает на их способность формировать коллаген. Установлена прямая корреляционная связь (rs = + 0,84) между содержанием пролина в стромальных фибробластах и числом эритроидных клеток-предшественников по данным миелограммы у больных ОЛЛ в фазу ремиссии.*

Выводы. *У детей с ОЛЛ стромальные фибробласты являются источником факторов, необходимых для функционирования гемопоэза, в частности эритроидных элементов, начиная с развития клеток-предшественников эритрона.*

The stromal microenvironment, which consists of closely related fibroblasts, macrophages and endothelial cells is a physiological niche for optimal functioning of hematopoietic elements, since their earliest stages of formation. Stromal fibroblasts collagen reproducing processes, maintain the structural integrity of connective tissue and are responsible for the state of hematopoietic microenvironment. The concentration of amino acids in stromal fibroblasts can influence the formation, recovery and functional properties of collagen. Changes in amino acids that form collagen, can lead to manifestations myelodysplasia, long-term recovery of bone marrow hematopoiesis after chemotherapy program and in cases of unfavorable acute leukemia in children, such as the development of relapses, complications, reducing the median survival of patients and others. Therefore, communication between parameters characterizing the quantitative and qualitative composition of stromal fibroblasts of bone marrow hematopoiesis and links in patients with acute leukemia, is relevant.

Aim. Determining the influence of structure and content of amino acids in stromal bone marrow fibroblasts grown in vitro, the state of hemopoiesis in children with acute lymphoblastic leukemia (ALL).

Material and methods. The study involved 46 patients with ALL aged from 4 to 12 years and 14 individuals without hematologic disease (control group). The survey was conducted in the I acute phase, phase of chemotherapy and remission. The efficiency of cloning fibroblast colony-forming units of bone marrow (ECFUf) was performed by the method of O.Ya.Fridenshteyn in V.S.Astakchov modification (2001). In patients with ALL the bone marrow obtained by sternal puncture was performed. The comparison group included patients who conducted surgical intervention on orthopedic pathology. Collection of material for culture studies conducted outside the inflammation and degenerative lesions sites.

Amino acid composition of stromal fibroblasts was determined in flushing of bone marrow CFUf from the culture medium. Cells were lysed by freezing and thawing. Stroma cells were separated from intracellular matrix contents by centrifugation. Obtained The precipitate (stroma cells) and supernatant substance (intracellular content that is a mixture of free amino acids) were obtained. The composition of free amino acids were determined by amino acid analyzer T-339. The elements myelogram were counted in the light microscope (increase x 900) after staining of bone marrow smears by Romanovsky – Himza. Processing of the material was held by the methods of mathematical statistics (Student , Spearman correlation coefficient).

Results and discussion. Studies were conducted in three phases. In the first phase the effectiveness of stromal fibroblast colony-forming units of bone marrow in children with ALL was studied. In the second step the amino acid

composition in vitro grown fibroblasts was determined. The third – the indicators of myelogram were defined, bone marrow cellularity, the percentage of erythroid and myeloid elements of hemopoiesis in children in these phase of leukemic process and these data there compared with fibroblasts amino acid composition.

The results of ECFUf research showed that in patients with ALL in all periods of observation the values were lower compared with healthy children, and especially in the acute phase of the disease (in 2,5–3,5 times) and in phase of chemotherapy (in 4,9-5, 2-fold), which may indicate the lower activity of the bone marrow stroma (Tabl. 1).

Table 1 – **Tests of ECFUf bone marrow of patients with ALL according to on the period of the disease (M±m)**

Groups of children / period ALL	ECFUf bone marrow among the 10^5 core containing cells
I acute period , n-46	19,1±2,4*, **
Period of chemotherapy, n-44	10,4±1,3*, **
Period of remission, n-27	28,7±2,3*
Children control group, n-14	52,3±3,2

Notes: * – the difference between the control group;
** – the difference between the acute phase and remission ($p<0,05$).

The individual fluctuations of bone marrow ECFUf were ranged in four graduations: lower 1,0; 1,0-20,0; 20,0-40,0 and higher than 40,0 in 10^5 nucleated cells. (tabl. 2).

Table 2 – **Distribution of patients for tests ECFUf bone marrow according to on the period of the disease (on a scale of ranks)**

Groups of children/ period ALL	ECFUf bone marrow among the 10^5 core containing cells			
	0,1–1,0	1,0–20,0	20,0–40,0	Higher 40,0
Children with ALL				
I acute period , n- 46	20	19	4	3
Period of chemotherapy, n-44	18	21	4	1
Period of remission, n-27	3	13	11	–
Children control group, n-14	–	2	12	–

Note. * – difference between the number of children within ECFUf rate compared to remission ($p<0,05$).

As seen from the table. 2, and the acute period and in phase of chemotherapy in the majority of patients (39 of 46 and 39 to 44, respectively) ECFUf values ranged from 0,1 to 20,0, while in remission period the majority of children (24 of 27) were in the range of 1,0 to 40,0 (in 10^5 c nucleated cells) (graduation I: 0,03-0,15-0,24, graduation II: 0,40-0,51-0,61). Regarding the comparison group of children, their rates were in the range of 20,0-40,0 (in 10^5 nucleated cells). Four patients had ECFUf higher than 40,0, all these children experienced unfavorable ALL course subsequently.

After the endpoint of chemotherapy program in manifestating of myelosuppression the most patients bone marrow ECFUf were increased but did not reach the standard values compared with healthy children.

Amino acid composition of bone marrow fibroblasts was studied in 27 samples of fibroblasts from patients with ALL in remission. The results revealed the presence of all amino acids, except oxyproline (Table. 3). This confirms the ability of fibroblasts to form collagen, and oxyproline absence (marker of collagen degradation) indicates the physiological process of collagen forming.

Regarding myelogram indicies in patients with initial ALL period, they showed that bone marrow cellularity ranged from 55,0 G/l to 160,0 G/l (mean 84,1±5,7 G/l) on the background of total lymphoblast infiltration (82,3±5,2%) (Table. 3). Under the chemotherapy the bone marrow cellularity (number мyelocariocytes) decreased dramatically to 17,3±3,1 G/l in the presence of tumor substrate eradication and the small number of erythroid and myeloid elements (up to 5,4% and 6,0% subsequently) were revealed. In remission time children demonstrated an almost complete recovery of erythroid and myeloid hematopoiesis branches.

Due to the low cellularity of erythroid and leukocyte hemopoiesis branches in initial period and chemotherapy period according to myelogram data, only the values defined in the remission phase were taken into account.

Table 3 – **Tests of myelogram in patients with ALL depending on the period of examination (M±m)**

Tests	I acute period, n-46	Period of chemotherapy, n-44	Period of remission, n-27
Myelocariocytes, G/л	84,1±5,7	17,3±3,1*	64,6±15,2
Lymphoblasts, %	82,3±5,2 *	1,8±0,5	0,7±0,3
erythroid elements, %	3,1±0,9 *	5,4±2,1*	36,4±8,5
myeloid elements, %	3,3±1,2 *	6,1±2,8*	43,1±9,4

Note. * – difference compared to remission ($p < 0,05$)

Taking into account the content of proline in fibroblasts, two groups of children were forming: the I group – proline was up to 1,0 mmol/ml, in the II group – higher than 1,0 mmol/ml. Distribution of patients in the groups was almost identical. In group I (n-12) proline into bone marrow fibroblasts averaged 0,98±0.21 mmol/ml in group II (n-15) – 0,99±0,23 mmol/ml. The percentage of bone marrow erythroid elements in I group of children was 18,5±1,8%, in the II group – 43,4±2,6%. Results of correlation analysis in children with ALL in remission established direct correlation between the number of erythroid progenitor cells in the bone marrow (proerytroblasts, erythroblasts and normosytes) and proline content in stromal fibroblasts of patients (r_s=+0,84). Between other amino acids that make up the stromal fibroblasts, bone marrow cellularity and the myeloid progenitor cells number in children with acute leukemia any correlation has not been established.

Thus, our findings suggest that stromal fibroblasts are the source of essential factors for the hemopoiesis functioning, in particular erythroid elements, starting from the development of erythron progenitor cells.

Conclusions

1. In patients with ALL in all periods of observation the efficiency of bone marrow stromal fibroblast colony-forming units cloning was lower compared with healthy children, and especially in the acute phase of the disease and chemotherapy phase, indicating a decrease in activity of the bone marrow stroma and disruption of collagen.

2. Amino acid profile of bone marrow stromal fibroblasts in patients with ALL during clinical hematologic remission showed the presence of the total spectrum of amino acids except oxyproline, indicating the ability of fibroblasts to form collagen.

3. The direct correlation (r_s =+0,84) between proline content in the stromal fibroblasts and the number of erythroid progenitor cells according myelogram in children with remission ALL is the evidence of bone marrow microenvironment have influence over the hemopoiesis processes.