

PECULIARITIES OF ABNORMAL KARYOTYPES FORMATION IN THERAPY-RELATED ACUTE LEUKEMIAS

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Aim: To determine ways of formation of abnormal karyotypes in two clinical cases of secondary acute leukemias of myeloid and lymphoid lineages. **Material and Methods:** Bone marrow cells of one patient with therapy-related acute monoblastic/monocytic leukemia and one patient with therapy-related acute lymphoblastic leukemia were examined by cytogenetic GTG banding technique. **Results:** An unusually large number of quantitative and structural anomalies of chromosomes in therapy-related acute monoblastic/monocytic leukemia have been established, which have many features in common with chromothripsis, namely instability of clones that manifested itself through quantitative anomalies (trisomy, monosomy, marker chromosomes, including chromosome 5), structural — t(9;11), deletions of the long arm of chromosomes 8 and 14, derivatives of chromosomes 3 and 7, ring chromosomes. In case of secondary acute lymphoblastic leukemia, the anomalous clone with balanced translocation in all 20 metaphase plates 46,XX,t(1;15)(p21;q24) has been registered, which is not described in the literature. Therefore, the diagnostic and prognostic value of such anomaly is unknown. **Conclusions:** Rearrangement with the involvement of the locus 11q23 was recorded in the case of chemotherapy treatment without topoisomerase II inhibitors. The complex karyotype formed after chemotherapy and radiotherapy, which is a criterion for an unfavorable prognosis of the disease, is considered as the equivalent of chromothripsis. t(1;15) is considered as an abnormality that can be attributed to the group of favorable secondary acute lymphoblastic leukemia prognosis. **Key Words:** therapy-related leukemia, chromosomal abnormalities, complex karyotype, prognostic significance, chromothripsis.

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Secondary leukemia is a fairly common pathological process that occurs over a period of time after the patient has undergone chemotherapy and/or radiotherapy for another cancer that has occurred in the past [1], including breast cancer [2], ovarian cancer [3] and non-Hodgkin's lymphomas [4].

Most secondary leukemias are myeloid [1, 5]. The WHO classification includes the term “therapy-related myeloid neoplasm” (t-MN) comprising t-myelodysplastic syndrome and therapy-related acute myeloid leukemia (t-AML). There are two subgroups of t-MN: 1) t-MN that follows treatment with alkylating agents and/or radiotherapy and 2) t-MN that follows treatment with DNA topoisomerase II inhibitors. t-AML following the treatment with alkylating agents usually develops in 5–7 years after the treatment, is preceded by t-myelodysplastic syndrome and often associated with unbalanced loss of genetic material, most often, deletion of chromosomes 5 or 7 or complex karyotype [5]. t-AML originating after treatment with DNA topoisomerase II inhibitors is characterized by a shorter latency period (1–3 years). Such t-AML is not preceded by myelodysplasia and usually associated with balanced chromosomal translocations relating to 11q23, *KMT2A* gene loci; however, occasionally there are cases without these changes [6]. Unlike secondary acute myeloid leukemia (s-AML) widely

reported in the literature and categorized in WHO classification of neoplasms of hematopoietic and lymphoid tissues [7], the secondary acute lymphoblastic leukemia (s-ALL) is much less known, primarily due to the rarity of this disease. According to the literature, the incidence of s-ALL is less than 1% of all acute leukemias and is from 2–3% [8] up to 9% of all acute lymphoblastic leukaemias [9]. Moreover, some s-ALL do not seem to be related to the history of prior therapy and some s-ALL cases are rather associated with the inherent predisposition of the patients [10].

In recent years, a new concept has been proposed for the transformation of a normal somatic cell into an abnormal one, called “chromothripsis”. The primary event of such transformation is the breaking of both DNA strands. At the cytogenetic level, the primary event is a balanced translocation, that is, the exchange of genetic material between two or more chromosomes, resulted in the formation of marker chromosomes at the final stage [11]. The phenomenon of chromothripsis has been described in solid tumors [12, 13], in neoplasms of hematopoietic and lymphoid tissues [14, 15], including acute myeloid leukemia [16, 17]. There are currently no standard definitions to identify chromothripsis, however, there are several suggestions, one of which includes the identification by FISH, SNP array, and sequencing [18]. Due to the similarity of the results of transformation of normal cells into anomalous, six molecular-genetic criteria were proposed to distinguish between the chromothripsis and the multi-blood process of genomic rearrangements [19].

The aim of the study was to analyze the features of the formation of an abnormal karyotype in bone marrow cells in two clinical cases of therapy-related acute leukemias.

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Abbreviations used: s-ALL – secondary acute lymphoblastic leukemia; s-AML – secondary acute myeloid leukemia; t-AML – therapy-related acute myeloid leukemia; t-MN – therapy-related myeloid neoplasm.

MATERIALS AND METHODS

The cytogenetic studies were performed on cells of heparinized bone marrow at the time of diagnosis induced acute leukemia. To increase proliferative activity, a suspension of leukemic bone marrow cells was cultured for 24 h in RPMI-1640 medium supplemented with 20% heat inactivated fetal calf serum, penicillin and streptomycin in a CO₂-incubator at 37 °C, 95% humidity and 5% CO₂. Metaphase chromosomes were prepared according to a commonly recognized technique [20] and stained by G-banding technique. Chromosomal abnormalities were detected according to International System for Human Cytogenetic Nomenclature 2016 [21]. The presence of chromosomal rearrangements in the abnormal clone was confirmed under conditions where two or more metaphase cells had identical anomalies or additional chromosomes, and when three or more metaphase cells had identical monosomies.

The patients gave written informed consent for the publication of their data analyses.

RESULTS AND DISCUSSION

Case 1. Patient L., 57 years old was hospitalized to surgical department in March 2017 with carbuncle of the chest wall. The peripheral blood test demonstrated the following: hemoglobin 85 g/l, erythrocytes $2.49 \cdot 10^{12}$, platelets $130 \cdot 10^9$, leukocytes $4.5 \cdot 10^9$, blasts 8%, myelocytes 2%, band neutrophils 29%, segmented neutrophils 17%, lymphocytes 35%, monocytes 8%. The surgery was performed. Then the sternal puncture demonstrated 30% of blast cells in bone marrow with negative myeloperoxidase, positive acid phosphatase and acid nonspecific esterase, and small granulated periodic acid-Schiff reaction. The acute monoblastic leukemia was diagnosed and the patient was further treated at the hematological department. According to the medical history, in 2010 the patient was treated for adenocarcinoma of the ovary and received postoperatively six cycles of chemotherapy with cisplatin and cyclophosphamide. Then after the recurrence in June 2011, three courses of chemotherapy with doxorubicin (total dose of 300 mg) and cisplatin + cyclophosphamide were provided. In January 2012, the disease progression was established, and additional three cycles of chemotherapy were performed by a similar scheme (end in April 2012). In May 2012, a pelvic recurrence without signs of spreading inside the intestine (according to laparoscopy and random biopsy) was ascertained, due to which a laparoscopic removal of the extracorporeal pelvic tumor was performed on May 23, 2012. According to the pathohistological study, the diagnosis of adenocarcinoma of the intestinal differentiation of G2-3 was established, and paclitaxel with carboplatin therapy was prescribed (end in October 2012). Then, in 2013–2017, until the diagnosis of acute monoblastic/monocytic leukemia, no adenocarcinoma specific therapy was received due to clinical and radiological remission (examination by computed tomography on 19.01.2017 did not reveal pathological changes).

Cytogenetic study revealed the presence of complex quantitative and structural abnormalities of chromosomes with formation of two clones:

44~47,XX,+3,der(3),der(3),-5,-6,der(7)?inv(7)(q22q33),del(8)(p23),t(9;11)(p22;q23),del(14)(q22),-18,-22,+r(?),+mar1,+mar2[cp17]/88~95<4n>,idem,+r(?)×4,+r(?)×4[cp3].

Both clones were composite. In the first clone, the number of chromosomes ranged from 44 to 47. Quantitative chromosome abnormalities include chromosome 5, 6, 18 monosomies, a ring chromosome formed from unspecified genetic material, and two varieties of marker chromosomes. Structural abnormalities of chromosomes are represented by the derivative of chromosome 7, which may have been formed as a result of paracentric inversion in the long arm involving disks 7q22 and 7q33; loss of genetic material in the short arm of chromosome 8 from disk 8p23 to 8pter; balanced translocation t(9;11)(p22;q23) and distal loss of genetic material in the long arm of chromosome 14 from disk 14q22 to 14qter (Fig. 1, 2).

In the second clone, doubling of quantitative and structural abnormalities of chromosomes was recorded and the appearance of four copies of the ring chromosome from the primary clone and four copies of the additional ring chromosome from the undetected genetic material (Fig. 3).

Translocation t(9;11)(p22;q23) is detected in 1–2% of cases of primary or t-AML (subtype acute mono-

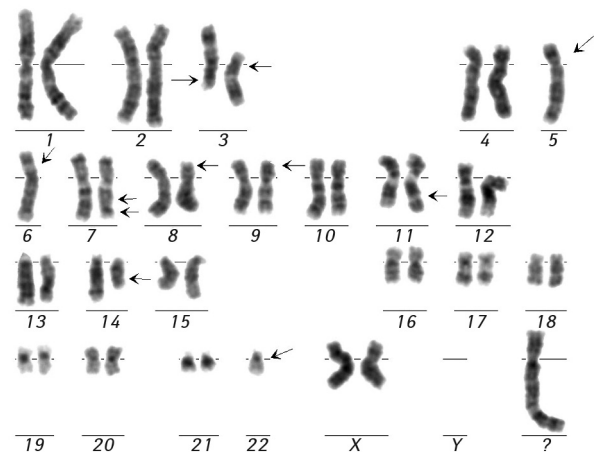


Fig. 1. Karyotype 44,XX,der(3),der(3),-5,-6,der(7)?inv(7)(q22q33),del(8)(p23),t(9;11)(p22;q23),del(14)(q22),-22,+mar1

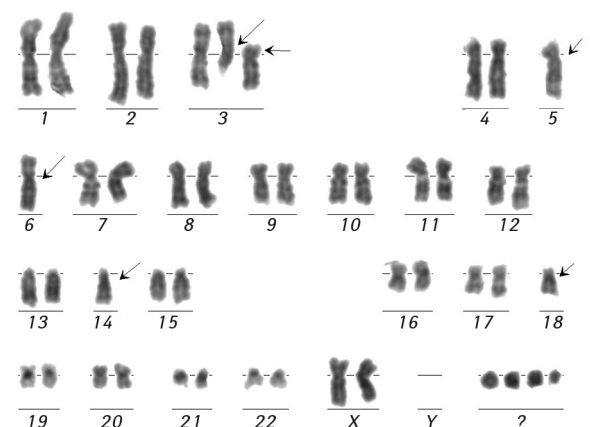


Fig. 2. Karyotype 46,XX,+3,der(3),der(3),-5,-6,-14,-18,+r(?),+mar2×2,+mar3

blastic, acute myelomonoblastic leukemias), accompanied by organomegaly, central nervous system lesions. It is interesting that the occurrence of this anomaly is not related to previous chemotherapy with topoisomerase II inhibitors [6, 10]. The prognostic value of t(9;11) ranges from intermediate to unfavorable [22, 23]. Monosomy of chromosomes 5, 6, 18, 22, as well as derivatives of chromosome 7 inv(7)(q22q33), deletions del(8)(p23) and del(14)(q22) can be considered as secondary anomalies of chromosomes that do not affect subtype of acute leukemia, but indicate a multilevel mechanism of formation of an abnormal clone. The appearance of ring chromosomes can lead to genetic instability of abnormal cells [24].

The presence of the second near-tetraploid clone led to a doubling of the quantitative and structural abnormalities of the chromosomes, which were recorded in the primary clone indicating a complication of this clone. In addition, in the near-tetraploid clone, additional chromosome abnormalities are recorded, which in turn, indicates further complication of clonal chromosome abnormalities. These additional chromosome abnormalities can contribute to the formation of anomalous clone resistance to chemotherapy.

As shown by M. Fontana et al. [17], a distinguishing feature of anomalous cells with chromothripsis is chromosomal instability, namely, changes in DNA repair and cell cycle, high level of single-nucleotide alterations, and, at cytogenetic level, the abnormalities involving the *TP53* gene, deletions 5q, complex karyotype, deletions of chromosomes 7, 12, 16, 17. FISH analysis shows the association of chromothripsis with the appearance of marker, derivative and ring chromosomes.

The findings we observed have many features in common with chromothripsis, namely, an instability of clones that manifested itself through quantitative abnormalities (trisomy, monosomy, marker chromosomes, including chromosome 5) and structural rearrangements: t(9;11), deletions of the long arm of chromosomes 8 and 14, derivatives of chromosomes 3 and 7, ring chromosomes. Notwithstanding, the presence of complications that led to the emergence of the composite karyotype may suggest that such cases need to be further investigated by SNP

array and eventual integration with DNA sequencing. We make the assumption that, despite the cause of the primary event, a similar pattern of cytogenetic rearrangements is observed at the final stage of development of the abnormal clone. Moreover, if there is confirmation of the presence of chromothripsis, it is possible to consider such complex karyotypes as the equivalent of chromothripsis.

Treatment of acute leukemia was carried out according to the protocol “7 + 3”, but remission was not achieved, the patient died from multiple organ failure, which developed on the background of infectious complications, toxic hepatitis and renal failure.

Case 2. Patient P., 66 years old was diagnosed with Cr gland mammaes, st. 2A, pT2N0M0 in May 2010. Surgical treatment was performed with removal of the left breast, clinical group 2. Morphological study of operative material revealed infiltrating ductal breast cancer with pronounced tumor tissue necrosis and the presence of secretions inside the ducts (carcinoma stage II of malignancy). Immunohistochemical reactions of abnormal cells with McAb to estrogen and progesterone receptors were negative, with polyclonal antibody to the *HER2/new* (*c-erbB-2*) oncogene, the abnormal cells have positive expression (1+). Subsequently, four courses of chemotherapy under the scheme FAC (S-1.8) with the use of 5-fluorouracil 800 mg per course, doxorubicin 80 mg and cyclophosphan 800 mg were held. No clinically significant toxicities were noted, and clinical and radiological remission was achieved. The patient was admitted to the hematological ward in November 2016 with complaints of weakness and shortness of breath. Analysis of peripheral blood showed: hemoglobin 33 g/l, erythrocytes $1.25 \cdot 10^{12}$, platelets $15 \cdot 10^9$, leukocytes $1.2 \cdot 10^9$, band neutrophils 1%, segmented neutrophils 21%, lymphocytes 24%, monocytes 15%.

In the bone marrow punctate, 86% of small-sized blast-like cells were counted, some of them are of the “hand mirror” type. Reaction to myeloperoxidase is negative, acidic phosphatase is moderate fine-granular and acidic nonspecific esterase — weak diffused. Blast cells immunophenotype CD45(dim)⁺CD34(bright)⁺CD7⁺⁺CD38⁺CD10⁺cyt3⁺TdT⁺CD5(dim)⁺CD3-CD4-CD8-CD16-CD56-CD20-CD19-CD14-CD13-CD15-CD33-CD79a-CD23-CD22-cytCD22-cytMPO⁻, which corresponds to the proT-acute lymphoblastic leukemia.

Objectively, the patient at the time of examination had the signs of severe hemorrhagic syndrome on the skin; albeit, liver, spleen and lymph nodes were not enlarged.

Results of karyotyping registered an anomalous clone with balanced translocation in all 20 metaphase plates analyzed: 46,XX,t(1;15)(p21;q24)[20] (Fig. 4). To date, such translocation has not been described in the literature [25]. Therefore, the diagnostic and prognostic value of such anomaly is unknown.

From November 2016, chemotherapy (Berlin-Frankfurt-Munster protocol) was initiated. The clinical and hematological remission after Unit I was achieved. Up to July 2019, the patient has completed treatment

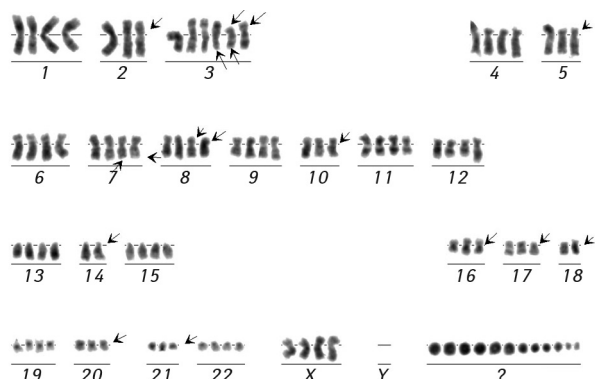


Fig. 3. Karyotype 95,XXXX,-2,+3x2,der(3)x2,der(3)x2,-5,der(7)?inv(7)(q22q33)x2,del(8)(p23)x2,del(14)(q22)x2,-18x2,-20,-21,+r(?)x4,+r(?)x4,+mar2x2

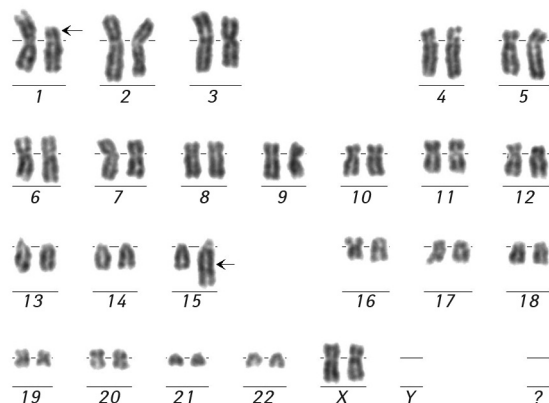


Fig. 4. Karyotype 46,XX,t(1;15)(p21;q24)

under the protocol, the patient condition is satisfactory, and remission persists. There are also no signs of breast cancer progression. Thus, translocation 46,XX,t(1;15)(p21;q24) may indicate a favorable prognosis of t-ALL.

The results indicate that the transformation of the normal karyotype of hematopoietic cells into abnormal, and, as a result, the development of t-ALL after the treatment of solid tumors with chemotherapy and radiotherapy, can develop in several ways.

To sum up, in some cases, there is a balanced exchange of genetic material within one chromosome or between two, and possibly more chromosomes. As a result, diploid chromosome level is recorded that is associated with a favorable prognosis. Otherwise, numerous quantitative and structural (balanced and unbalanced) rearrangements of chromosomes occur, and marker chromosomes are formed leading to the formation of a hyperdiploid karyotype associated with unfavorable prognosis requiring radical first-line treatment, including allogeneic stem cell transplantation.

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