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Copy Number Aberrations Landscape of a Breast Tumor, Connection with the Efficiency of Neoadjuvant Chemotherapy

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Abstract. The research involved 80 patients diagnosed with breast cancer (BC). Each patient had their tumor biopsy material sampled before their treatment. We studied the tumor tissue using the CytoScan HD Array (Affymetrix, USA) microarray to evaluate the CNA landscape. We studied the frequency of segmental and numerical CNA occurrence, their association with the efficiency of neoadjuvant chemotherapy (NAC). We found that the biggest number of amplifications (with frequency over 60%) were found in the following locuses: 1q32.1 1q32.3, 1q42.13, 1q42.2, 1q43. The biggest frequency of deletions (more than in 58% of the patients) was found in these locuses: 16q21, 16q23.2, 16q23.3, 17p12, 17p13.1. However, we found the locuses with full absence of segmental chromosome anomalies. We observed trisomy most frequently in the 7, 8, 12, and 17 chromosomes, and monosomy in the 3, 4, 9, 11, 18, and X-chromosomes. We demonstrated the connection between the high frequency of cytobands with CNA in the patients' tumors and the efficiency of NAC. We also identified the cytobands, whose CNA are linked to the response to NAC.

INTRODUCTION

Deletions and amplifications of chromosome regions and individual chromosomes are called Copy Number Aberrations (CNA). These types of cytogenetic disorders may affect the expression of genes; generally, in cases of deletions, the expression of genes located in the deleted regions is decreased, and it is increased in the cases of amplifications [1, 2]. CNA are especially widespread in solid tumors of various localization, namely in breast tumors. CNA is most frequently observed in the 1q, 8q, and 16q regions of a breast tumor. The amplifications are noted in 1q, 8p12, 8q24.21, 17q12, 11q13.3, and 11q13.5, involving such known oncogenes as HER2, c-Myc, CCND1, and PAK1; deletions are noted in the 8p, 11q, 16q, and 17p chromosome locuses, where in most cases the tumors suppress genes are located [3]. There are the data on the connection between the CNA of tumor DNA with the molecular subtypes of BC and expression features of a tumor [4–6]. For example, the amplifications of the 1q21-43 and 16p12 chromosome regions and deletion of 16q21-q24 are connected with the presence of estrogen receptors on the tumor cells, but the deletions of the 4p13-16 and 5q11.2-q31 sites are associated with the absence of progesterone receptors. Various BC tumor clones may be characterized by the specific structural chromosomal and numerical aberrations. They constitute the general state of the CNA landscape and they develop as a result of one or several cycles of clonal expansion. We may suggest that the presence of certain chromosome anomalies in a tumor before treatment can be the decisive factor in the progression of a tumor and its sensitivity to treatment.
The quantity of the studies dedicated to the connection between CNA and NAC in BC is insufficient. In the case of no effect by NAC with taxanes, Korean researchers found the amplifications in the 8q (24.3, 24.22, 22.1-3), 13q21.1, and 20q (13.2-13.33) regions and deletions of the 8p23.3-1 and 17p13.3 sites [7]. The increased level of CNA determines the inefficiency of taxane treatment and enhances the sensitivity to the platinum medication [8]. Thus, studying CNA landscape of a breast tumor before treatment and evaluating the connection with the efficiency of NAC are currently important.

MATERIAL AND METHODS

We examined 80 BC patients with the morphologically verified diagnosis on the IIA–IIIB (T1-3N0-3M0) stages, aged 28–68 (average age: 48.2 ± 2.4 years old), who were being treated at the clinics of Cancer Research Institute, Tomsk National Research Medical Center. The research was performed in accordance with the Declaration of Helsinki (1964) and with the approval of the institute ethics committee. All patients signed the informed consent forms for the study. The patients received 2–4 courses of chemotherapy according to the FAC, CAX regimens or taxotere monotherapy in the neoadjuvant mode. 3–5 weeks after the NAC the patients underwent radical or subcutaneous mastectomy, radical resection, sectoral resection, or some other type of breast-conserving surgery, then the patients received two courses of FAC adjuvant chemotherapy, radiotherapy, and/or hormonal treatment according to the recommendations. The efficiency of preoperative chemotherapy was evaluated according to the WHO and the Union for International Cancer Control [9] criteria with the help of ultrasound imaging and/or mammography, which were performed before the treatment, after 2 courses of NAC, and before the surgery. According to the international recommendations, we formed the groups of the patients whose tumors stabilized or progressed after the preoperative chemotherapy (NAC no-response group), and the patients with partial regression (positive response group) [10].

The material for the study was the biopsy samples taken before the treatment. Tumor biopsy sampling was performed with the help of ultrasound-guided pistol biopsy. We extracted DNA from the breast tumor tissue with the help of the QIAamp DNA mini Kit (Qiagen, Germany) according to the recommendations by the manufacturer. The concentration and purity of the DNA extracted were evaluated on the NanoDrop-2000 spectrophotometer (Thermo Scientific, USA). The concentration amounted from 50 to 150 ng/μl, A260/A280 = 2.10–2.35; A260/A230 = 2.15–2.40.

We performed microarray analysis using the CytoScanTM HD Array high-density microchips manufactured by Affymetrix (USA). This microarray contains 2,670,000 markers—1,900,000 non-polymorphic markers, and more than 750,000 single nucleotide polymorphisms (SNP), which enable determining the structural variations of more than 36,000 genes, namely the CNA. The procedures of sample preparation, hybridization, and scanning were performed according to the protocol of the manufacturer using the Affymetrix GeneChip® Scanner 3000 7G system (Affymetrix, USA). We used the “Chromosome Analysis Suite 3.0” application (Affymetrix, USA) to process the results of microarray analysis.

RESULTS

At the first stage, we analyzed the frequency of segmental chromosome anomalies for each chromosome in each patient. Figure 1 represents the frequencies of the amplification and deletion occurrences for each of the 862 cytobands of all chromosomes in the treated patients.

We found that the frequency of CNA occurrence strongly varies for different chromosomes. The study showed that the highest frequency of amplifications (more than 60.0% of the patients) was detected on the long arm of the 1 chromosome in the following locuses: 1q32.1, 1q32.2, 1q32.3, 1q42.13, 1q42.2, 1q43. The biggest frequency of deletions (more than in 58.0% of the patients) was found in these locuses: 16q21, 16q23.2, 16q23.3, 17p13.1, 17p12. We also found the locuses with the complete absence of segmental chromosome anomalies—the absence of the amplifications is the case for the 4p13, 13p13-13p11.1, 14p13-11.1, 14q11.1, 15p13-11.1, 15q11.1, 21p13-11.1, 21q11.1, 22p13-11.1 locuses; the deletions are absent in the 1q23.2, 1q25.3, 8q23.2, 8q23.3, 8q24.11, 13p13, 13p12, 13p11.2, 13p11.1, 14p13, 14p12, 14p11.2, 14p11.1, 14q11.1, 15p13, 15p12, 15p11.2, 15p11.1, 15q11.1, 21p13, 21p12, 21p11.2, 21p11.1, 21q11.1, 22p13, 22p12, 22p11.2, 22p11.1 locuses. We also found the regions with neither deletions nor amplifications: 13p13-13p11.1, 14p13-11.1, 14q11.1, 15p13-11.1, 15q11.1, 21p13-11.1, 21q11.1, 22p13-11.1. Also we calculated the numerical chromosome anomalies. We observed trisomy the most frequently in the 7, 8, 12, and 17 chromosomes, and monosomy in the 3, 4, 9, 11, 18, and X-chromosomes.
The frequency of cytobands with CNA (as related to the total number of cytobands) in some patients’ tumors also varied significantly: from 1 to 608, which constitutes from 0.1% to 75% of the total number of cytobands. One may talk of the CNA frequency continuum: there are the patients, who may have extensive deletions or amplifications in each chromosome, as other ones do not have them almost completely.

The second stage was studying the association of the response to NAC with the frequency of CNA occurrence. The results showed that presence of an objective response to NAC (partial or complete regression) was observed in the cases of bigger numbers of amplifications and deletions (Fig. 2).

82% of the group of the patients with fewer than 60 cytobands with CNA manifested the absence of response to NAC (stabilization or progression). 41% of the patients demonstrated a high level of CNA, and the frequency of cytobands with the deletions or amplifications amounts to more than 300. 73% of these patients responded to NAC. It is possible to suggest that a significant imbalance of cell signatures in a tumor, which is connected with a big number of CNA and with the development of genetic instability, contributes to a more substantial cytotoxic effect of the conventional chemo-medication used in treatment. First of all, this is true for the medications which affect the DNA molecule (cyclophosphan, fluorouracil, xeloda, platinum medication). This statement is supported by S.E. McClelland et al., who showed the correlation between the increased level of CNA with the resistivity to taxanes and the sensitivity to the platinum medication [8].

Then all patients were divided into two groups depending on their response to NAC: group 1—the patients with no response to NAC (stabilizations of progression of tumor process after NAC), group 2—the patients with objective response to NAC (partial or complete regression of tumor after treatment).


The locuses with simultaneous absence of CNA are: 13p13-11.1, 14p13-11.1, 14q11.1, 15p13-11.1, 15q11.1, 21p13, 21p12, 21p11.2, 21q11.1, 22p13-11.1. This being said, the maximum frequency of amplifications occurrence (48.3% or more) was detected in the 8q22.3, 8q23.2, 8q23.3, 8q24.13 regions.

It is worth noting that the highlighted locuses with high frequency of amplifications occurrence showed the absence of deleted regions.

FIGURE 1. The frequency of CNA occurrence in each of the 862 cytobands of the chromosomes in the patients included in the study. The horizontal axis—the cytobands and chromosomes from 1 to X; the vertical axis—the frequencies of the deletions (below the X-axis) and amplifications (above the X-axis) for each cytoband.
FIGURE 2. Connection of NAC effect and frequency of cytobands with CNA in a breast cancer tumor before treatment. Horizontal axis—frequency of cytobands with CNA, vertical axis—frequency of response to NAC (square markers) or absence thereof (triangle markers).

FIGURE 3. CNA occurrence frequency in the patients with stabilizing or progressing. The horizontal axis—the cytobands and chromosomes from 1 to X; the vertical axis—the frequencies of deletions (below the X-axis) and amplifications (above the X-axis) for each cytoband.

The biggest amount of deletions (44.8–48.3% and more) in this group was detected in the 8p23.3, 8p21.3, 8p21.2, 16q21, 17p13.1, 17p12, 17p11.2 cytobands.


The maximum frequency of amplifications occurrence (60.0% and more) was detected in the long arm of the 1 chromosome 1q21.3-44 and in the cytobands. The maximum number of deletions (60.0% and more) for this group was detected in the 11q22.3-23.3, 16q12.2, 16q21-24.2, 17p13.3-11.2 locuses.

During the joint analysis of the two groups, we found the cytobands, in which the difference between the frequencies of chromosome anomaly occurrence for the groups with or without objective response to NAC reached a maximum value of 35% and more. The biggest difference in the frequency of amplification occurrence between the groups was shown on the long arm of the 1 chromosome 1q23.1-44, and the biggest difference in the frequency of deletion occurrence between the groups was in the 11q22.1-23.2, 16q22.2, 16q22.3, 16q23.1, 18p11.21 regions.

We also calculated the numerical chromosome anomalies. This data may be used to predict the efficiency of NAC. The patients with amplifications on the long arm of the 1 chromosome and/or deletions in certain cytobands of the 11, 16, 18 chromosomes are more likely to respond to NAC.
FIGURE 4. The frequency of CNA occurrence in the patients with partial or complete regression of tumor. The horizontal axis—the cytobands and chromosomes from 1 to X; the vertical axis—the frequencies of deletions (below the X-axis) and the amplifications (above the X-axis) for each cytoband.

Thus, as a result of the study, we described the CNA landscape of breast tumor before treatment for the general group of patients, and performed the same separately for the patients with significant response to NAC and absence thereof. We described the frequencies of all cytobands occurrence, found the cytobands with the highest frequency of CNA occurrence and the absence of deletions or amplifications, as well as numerical CNA. We demonstrated the connection between the high frequency of cytobands with CNA in patients' tumors and the efficiency of NAC. We also identified the cytobands, whose CNA are linked to the response to NAC.

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