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Aberrations of the number of copies (CNA) in the genome of luminal B breast tumor

Ibragimova M.K.[1], Tsyganov M.M.[1], Slonimskaya E.M.[2], Litviakov N.V.[1]

[1] *Cancer Research Institute, Tomsk National Research Medical Center of Russian Academy of Sciences*
5, Kooperativny Str., 634009, Tomsk, Russian Federation

[2] *Saint-Petersburg State University*
7-9, University Emb., 199034, Saint-Petersburg, Russian Federation

ABSTRACT

Aim. To describe the CNA (Copy Number Aberration) landscape of luminal B breast tumor before treatment.

Materials and methods. The study included 100 patients with breast cancer (BC) of luminal B subtype for which a biopsy of the tumor material was performed prior to neoadjuvant chemotherapy (NAC). The tumor DNA was examined using a CytoScan HD Array microarray (Affymetrix, USA). The obtained microarray data were correlated with NAC efficacy.

Results. The study showed that loci 1q32.1–32.3, 1q41–42.2, and 8q24.21 had the highest frequency of amplifications (in more than 65% of patients). The highest deletion frequency (in more than 60% of patients) was found in loci 16q21, 16q22.1, 16q23.1–24.1, 17p13.1, and 17p12. Trisomy was most often observed in chromosomes 7, 8, 12, and 17, and monosomy in chromosomes 3, 4, 9, 11, 18, and X-chromosomes. The CNA landscape of luminal B subtype breast tumors is different from triple-negative breast cancer. The largest difference in the frequency of amplifications between patients with an objective response to NAC and patients with no response to NAC was shown in 1q24.2–42.2 loci (46%), and the largest difference in the frequency of deletions (more than 30%) between groups was in regions 6q16. 3, 11p15.4, 11q23.1, and 16q22.2–22.3. These loci can be considered potential predictive markers.

Conclusion. The research determined loci with the highest amplification and deletion frequencies for luminal B breast cancer. Potential predictive markers for the given molecular subtype were identified.

Key words: breast cancer, microarray analysis, deletions, amplifications, neoadjuvant chemotherapy.

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. The study was carried out in compliance with the ethical standards developed in accordance with the Helsinki Declaration of the World Medical Association Ethical Principles for Conducting Scientific Medical Research with Human Participation as amended in 2000 and the Rules of Clinical *Practice in the Russian Federation*, approved by the Order of the Ministry of Health of the Russian Federation of 19.06.2003, No. 266. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at Cancer Research Institute, Tomsk National Research Medical Center.

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✉ *Ibragimova Marina K.,* e-mail: imk1805@yandex.ru.

Аберрации числа копий в геноме опухоли молочной железы люминального подтипа B

Ибрагимова М.К.[1], Цыганов М.М.[1], Слонимская Е.М.[2], Литвяков Н.В.[1]

[1] Научно-исследовательский институт (НИИ) онкологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634009, г. Томск, пер. Кооперативный, 5

[2] Санкт-Петербургский государственный университет (СПбГУ)
Россия, 199034, г. Санкт-Петербург, Университетская набережная, 7-9

РЕЗЮМЕ

Цель. Описание ландшафта Copy Number Aberration (CNA) опухоли молочной железы люминального подтипа B до лечения.

Материалы и методы. В исследование включены 100 больных раком молочной железы (РМЖ) люминального подтипа B, для которых проведен забор биопсийного материала опухоли до проведения неоадъювантной химиотерапии (НХТ). ДНК из опухоли исследована при помощи микроматрицы CytoScan HD Array (Affymetrix, США). Полученные микроматричные данные соотнесены с эффективностью НХТ.

Результаты. Показано, что наибольшая частота амплификаций (более чем у 65% больных) наблюдается в следующих локусах: 1q32.1-32.3, 1q41-42.2, 8q24.21. Наибольшая частота делеций (более чем у 60% больных) была обнаружена в локусах 16q21, 16q22.1, 16q23.1-24.1, 17p13.1, 17p12. Трисомия чаще всего наблюдалась в 7-, 8-, 12- и 17-й хромосомах, моносомия – в 3-, 4-, 9-, 11-, 18-й и X-хромосомах. Ландшафт CNA опухоли молочной железы люминального подтипа B отличается от трижды негативного РМЖ. Наибольшая разница частоты встречаемости амплификаций между больными с объективным ответом на НХТ и больными с отсутствием ответа на НХТ показана в 1q24.2-42.2 локусах (46%), а наибольшая разница частоты встречаемости делеций (более 30%) – между группами в регионах 6q16.3, 11p15.4, 11q23.1, 16q22.2-22.3. Данные локусы могут быть рассмотрены в качестве потенциальных предиктивных маркеров.

Заключение. Установлены локусы с наибольшей частотой амплификаций и делеций для рака молочной железы люминального подтипа B. Идентифицированы потенциальные предиктивные маркеры для данного молекулярного подтипа.

Ключевые слова: рак молочной железы, микроматричный анализ, делеции, амплификации, неоадъювантная химиотерапия.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Thanks to the Cancer Genome Atlas Program (TCGA), it became clear that tumors of many localizations contain not only point mutations of oncogenes and tumor suppressor genes but also a large number of various chromosomal abnormalities that play a key role in carcinogenesis and tumor progression [1]. The most common chromosomal abnormalities are found in solid tumors [2]. Deletions or amplifications of chromosomal regions and individual chromosomes are referred to as aberrations of the number of DNA copies or CNA (Copy Number Aberration). These types of cytogenetic disorders can affect gene expression; as a rule, in deletions, the expression of genes localized in the deleted region is reduced, while in amplifications it is increased [3]. Breast cancer (BC) is no

exception. Despite the fact that breast tumors have a high degree of intra-tumoral heterogeneity, the most common chromosome aberrations have been identified for them. In breast cancer (according to TCGA data), CNA in 1q, 8q, 8p, 11q, 13q, 16q, 17q and 20q regions have a high frequency of occurrence [4–6].

To date, the detailed elaboration of changes in tumors has begun and CNA genetic characteristics of specific tumor localizations and related patterns of gene expression are being described. In fact, CNA tumor-specific landscapes with large changes in the number of genomic copies lead to global deregulation of tumor cell transcriptomes. In addition, the molecular characterization of cytogenetic abnormalities has made it possible to gain insight into the mechanisms of oncogenesis and in some cases has led to the clinical implementation of effective diagnostic and prognostic tools, as well as treatment strategies aimed at a specific genetic anomaly.

The study by J.Y. Goh et al. determined that amplification of 1q21.3 chromosome is a new biomarker and an effective target for breast cancer. This amplification is present in 10–30% of primary tumors, and in more than 70% of recurrent tumors, regardless of breast cancer subtype. The molecular mechanism by which 1q21.3 amplification is associated with a relapse of breast cancer, including the functional relationship between S100A7/8/9 and IRAK1, was investigated. Using ddPCR, the authors developed a molecular analysis based on a blood test to detect 1q21.3 amplification in extracellular DNA and showed that this amplification can serve as a circulating biomarker to predict early relapse and monitor the breast tumor response to chemotherapy [7].

Currently, there are studies of the CNA association and clinical and morphological parameters of the tumor for individual subtypes of breast cancer, in particular, for triple negative breast cancer. According to these studies, 10p and 12q chromosomal regions show the highest amplification frequency, which corresponds to an increase in the number of copies of the GATA3 and MDM2 genes. Amplifications in chromosomes 1q (MDM4), 3q (PIK-3CA), 6p (CCND3), 8q (MYC) and 18 (BCL2 and SMAD4) are less frequent, and frequent deletions include chromosomes 4p (FGFR3), 5q (PIK3R1), 8p (DBC2), 9p (NR4A3), 12 (MDM2) and 22 (CHEK2) [8].

However, most studies do not determine the CNA of the entire genome, investigating only the key genes involved in tumor pathogenesis. No full-genome CNA landscape description is provided for luminal B subtype of breast cancer.

The study aims to describe the CNA landscape of luminal B breast tumor before treatment.

MATERIALS AND METHODS

In the course of this study, a bank of biological material was collected from 100 patients with a morphologically verified diagnosis of luminal breast cancer (11 of them are luminal B HER2+) and a detailed register of clinical and morphological data was compiled (average age 46.2 ± 0.4 years) (Table 1). Biopsy material was collected from each patient before treatment with the help of ultrasound-guided pistol biopsy. DNA was isolated from samples using the QIAamp DNA miniKit kit (Qiagen, Germany) in accordance with the manufacturer's instructions.

Table 1

Clinical and morphological parameters of the examined patients with breast cancer

Clinical and morphological parameters		Number of patients (%)
Age, years	≤45	41 (41%)
	>45	59 (59%)
Menstrual status	Premenopause	58 (54%)
	Postmenopause	42 (42%)
Histological type	Invasive ductal carcinoma	85 (85%)
	Invasive lobular carcinoma	8 (8%)
	Medullary carcinoma	1 (1%)
	Other types	7 (7%)
Tumor size	T_1	13 (13%)
	T_2	78 (78%)
	T_3	4 (4%)
	T_4	5 (5%)

Table 1 (continued)

Clinical and morphological parameters		Number of patients (%)
Lymph node metastasis	N_0	45 (45%)
	N_1	43 (43%)
	N_2	4 (4%)
	N_3	8 (8%)
Molecular subtype	Luminal B	100 (100%)
Epidermal growth factor receptors HER2	0/+	89 (89%)
	++/+++	11 (11%)
Histological form	Unicentric	70 (70%)
	Multicentric	30 (30%)
NAC regimen	CAX	19 (19%)
	FAC/AC	31 (31%)
	Taxotere	20 (20%)
	AT/ACT	9 (9%)
	CP	12 (12%)
	Not carried out	9 (9%)
Response to NAC	Progression	4 (4%)
	Stabilization	22 (22%)
	Partial regression	53 (53%)
	Complete regression	12 (12%)
	Not carried out	9 (9%)

Note. NAC – neoadjuvant chemotherapy; CAX – cyclophosphamide, adriamycin, xeloda; FAC – 5-fluorouracil, adriamycin, cyclophosphamide; CP – cyclophosphamide, cisplatin; AT – adriamycin, docetaxel; AC – adriamycin, cyclophosphamide; ACT – adriamycin, cyclophosphamide, docetaxel.

To study CNA in tumor cells, a high density microarray CytoScan HD Array (Affymetrix, USA) was used, which allows a full-genome format to evaluate DNA deletions and amplifications in all tumor cells at the same time and quantitatively analyze the representation of mutation (or a clone carrying mutation) against normal DNA.

The effectiveness of pre-surgery chemotherapy was evaluated according to the criteria of the WHO and the International Union Against Cancer with the help of ultrasound examination and / or mammography. Full regression (100% decrease in tumor), partial regression (decrease in tumor volume by more than 50%), stabilization (decrease in tumor volume by less than 50% or increase by no more than 25%) and progression (increase in tumor volume by more than 25%) were recorded. According to international recommendations, during pre-surgery chemotherapy breast cancer patients with stabilization or progression constituted a group with no response to NAC, and patients with partial and complete regression formed a group with an objective response. The program "Chromosome Analysis Suite 4.0" (Affymetrix, USA) was used to process the results of microchipping (bioinformatic analysis).

RESULTS

The first stage saw the analysis of CNA occurrence frequency carried out for all 862 cytobands for each patient included in the study. The amplification and deletion frequencies are shown in Figure 1 and in the appendix in the form of tabular data. Table 2 presents data on the genomic regions with a high incidence of CNA and their absence in the group of breast cancer patients.

In most cases, amplifications and deletions were absent in the pericentromeric regions of chromosomes 13, 14, 15, 21, and 22. Moreover, the study showed that the absence of amplifications in loci 10q23.32–24.33, 11q23.1–23.2, and 13q14.11–14.3 was accompanied by the presence of deletions in more than 30% of patients, and, conversely, the absence of deletions in loci 8q12.1 and 8q24.11–24.21 was accompanied by the presence of amplifications in more than 40% of patients (Fig. 1, appendix). Numerical chromosomal abnormalities were calculated. Trisomy was most often observed in chromosomes 7, 8, 12, and 17; monosomy was most often observed in chromosomes 3, 4, 9, 11, 18 and X chromosomes.

Next, we studied the association of the response to NAC with the CNA occurrence frequency. Before treatment patients were divided into two groups: group

1 included patients with stabilization and progression of the tumor process after NAC (n = 26), while group 2 consisted of patients with partial and complete tumor regression after treatment (n = 65).

Table 2

Data on the genomic regions with a high incidence of CNA and their absence in the group of breast cancer patients

Parameter	Locus
Frequency of amplifications >65%	1q32.1-32.3, 1q41–42.2, 8q24.21
Frequency of deletions >60%	16q21, 16q22.1, 16q23.1–24.1, 17p13.1, 17p12
Absence of amplifications	10q23.32–24.33, 11q23.1–23.2, 13p12–11.1, 13q14.11–14.3, 14p13–11.1, 14q11.1, 15p13–11.1, 15q11.1, 21p13–11.1, 21q11.1, 22p13–11.1
Absence of deletions	8q12.1, 8q24.11–24.21, 13p13–11.1, 14p12–11.1, 14q11.1, 15p12–11.1, 15q11.1, 21p13–11.1, 21q11.1, 22p13–11.2
Absence of amplifications and deletions	13p13–11.1, 14p12–11.1, 15p12–11.1, 21p13–11.1, 21q11.1, 22p13–11.2

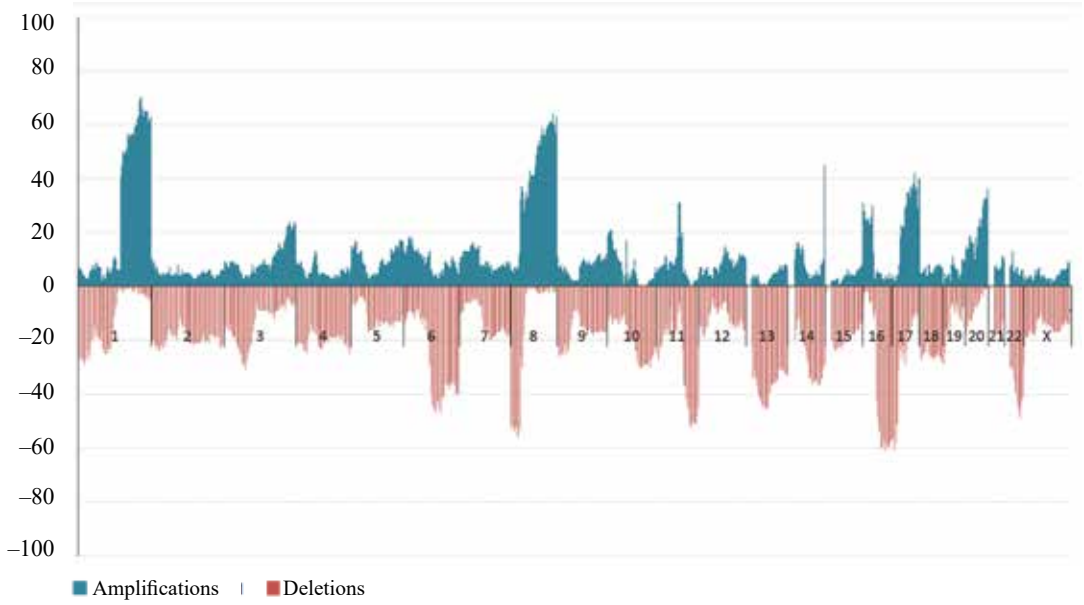

Fig. 1. CNA frequency in a luminal B molecular subtype breast tumor

The highest frequency of amplifications (over 60%) in the group of patients with stabilization and progression after NAC was found only in loci 8q23.1–24.3. It is interesting to note that in the presence of more than 60% of amplifications in regions 8q23.1–24.3, there was a complete absence of deleted sites in these loci. The maximum deletion rate (more than 50%) in group 1 was observed in loci 8p23.3, 16q21, 16q23.1–24.2, and 17p13.3–11.2. At the same time, locus 8p23.3 with the highest deletion frequency demonstrated the absence of amplifications. A general picture of the incidence of CNA in patients with stabilization and progression of the tumor process is presented in Figure 2.

For the 2nd group of patients, the highest amplification frequency (84%) was found in locus 1q32.2. The amplification frequency of more than 60% was found in the long arm of chromosome 1, loci 1q23.2–25.3 and 1q31.1–44, and the long arm of chromosome 8, 8q22.1–24.3. At the maximum frequency of amplifications in these regions, deletions were practically absent. The maximum deletion rate (72%) was observed in locus 17p13.1. A deletion rate of more than 50% was found in a large number of loci: 6q14.1–16.3, 6q21–22.1, 8p23.3–21.1, 11q21–25, 13q14.11–14.3, 13q21.1, 16q11.2–13, 16q21–24.3, 17p13.3–11.2, and 22q12.3–13.2. A general picture of the CNA incidence in patients of group 2 is also presented in Figure 2.

In the joint analysis of the two groups, cytobands were found in which the difference in the frequency of occurrence of chromosomal abnormalities in the groups with the presence and absence of an objective response to NAC reached a maximum value of 30% or more. The largest difference in the frequency of occurrence of amplifications between groups is shown

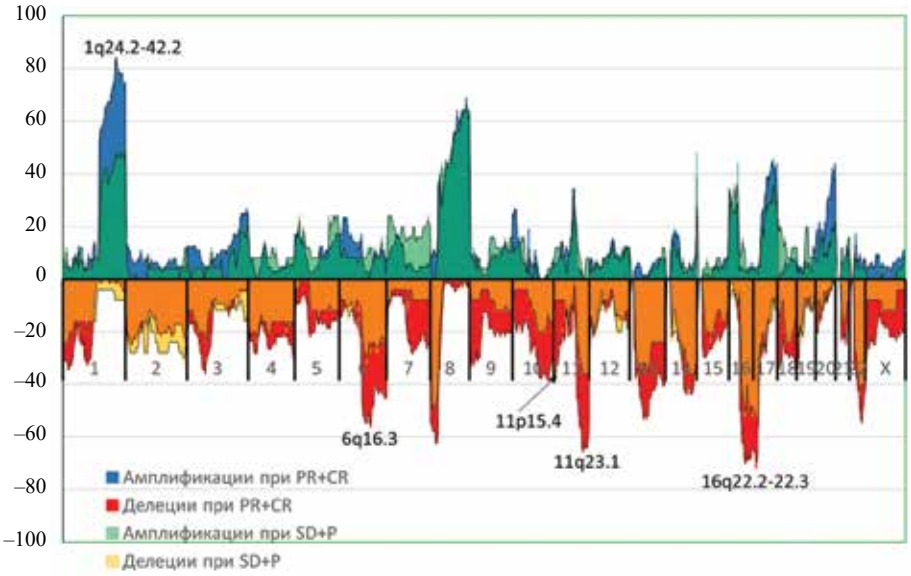

Fig. 2. The ratio of amplification and deletion frequencies in the tumor before treatment, depending on the effect of neoadjuvant chemotherapy: loci with the largest difference in amplification and deletion frequencies are signed. SD + P – stabilization + progression; PR + CR – partial regression + complete regression

in loci 1q24.2–42.2 (46%), and the largest difference in the frequency of occurrence of deletions (more than 30%) between groups is in regions 6q16.3, 11p15.4, 11q23.1, 16q22.2–22.3 (Fig. 2). These loci have potential predictive significance for luminal breast cancer, which must be validated in prospective studies.

DISCUSSION

Given the fact that breast cancer is a genetically heterogeneous disease, it is now necessary to conduct studies to identify the spectrum of molecular and genetic features of this tumor in order to develop new approaches to the treatment of breast cancer patients. Therefore, features of the genetic landscape of the breast tumor need to be described in detail, with division into molecular subtypes and based on the main clinical indicators.

Currently, data on the frequency analysis of chromosomal aberrations for a small sample of patients ($n = 12$) with triple negative breast cancer have already been published. Microarray analysis determined chromosome regions with the most frequent amplifications (1q, 3q, 6p, 8q), frequent trisomy of chromosome 18, regions with the most frequent deletions (4p, 5q, 8p, 9p) and monosomy of chromosomes 12 and 22. Moreover, many unique amplifications that occurred exclusively in individual patients were identified [8].

Similar data were obtained by Matthew D. Burstein et al. in a large sample of patients ($n = 278$) with triple negative breast cancer. Thus, the features of CAN occurrence frequency were characterized, which show that chromosomes 1q31.2, 3q26.1 and 8q23.3 demonstrated the highest frequency of amplification occurrence (more than 84%), and the highest deletion frequency was found in chromosomes 8p23.2, 9p21.3 and 10q23.31 [9]. In contrast to triple negative breast cancer [9], in the case of luminal B breast cancer the telomeric part of the long arm of chromosome 1 has a high frequency of amplifications, but in 3q26.1 the frequency of amplifications is much lower, and in the long arm of chromosome 8 the highest frequency (66%) is observed in 8q24 .21, where one of the most famous oncogenes, -c-MYC, is located. Triple negative and luminal B subtype breast cancer also differ in loci with the highest deletion frequency (Table 2), in particular, loci in the long arm of chromosome 16 and the short arm of chromosome 16 (17p13.1, 17p12) are most often deleted in luminal B BC. Locus 17p13.1 contains one of the most famous tumor suppressor genes TP53. These data indicate that the CNA landscape of breast tumor is dependent on its molecular subtype.

In addition to the description of the CNA landscape itself, it is important to understand that such data can form the basis to develop new markers of treatment efficacy for patients with breast pathology. The study by Kazantseva et al. examined molecular and genetic markers of effectiveness of neoadjuvant chemotherapy with anthracyclines in patients with breast cancer, where a sample of 46 patients with breast cancer showed that deletions of 18p.11.21; 11q22.1 and amplifications of loci 1q24.1–43 can be considered

as predictive criteria for high efficiency of NAC. The presence of at least one of the markers makes it possible to predict a high efficacy of pre-surgery treatment with anthracyclines in 85.3% of cases [10]. In the present study on luminal B subtype breast cancer, the long arm of chromosome 1 in patients with an objective response also showed a relatively high frequency of amplifications. At the same time, deletions were more often observed in patients with an objective response in loci 6q16.3, 11p15.4, 11q23.1, 16q22.2 – 22.3, which differs from the work of Kazantseva and her co-authors.

CONCLUSION

The study described the CNA landscape of luminal B breast tumor before treatment as well as the CNA landscape in patients with an objective response to NAC and its absence. The incidence rates of aberrations in all cytobands were established; aneuploidy and cytobands with the highest frequency of CNA occurrence and their absence were detected. Differences in the CNA landscape of luminal B subtype breast tumor and triple negative breast cancer were discussed.

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Authors information

Ibragimova Marina K., Junior Researcher, Laboratory of Oncovirology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk. ORCID 0000-0001-8815-2786.

Tsyganov Matvey M., Cand. Sci. (Biology), Senior Researcher, Laboratory of Oncovirology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk. ORCID 0000-0001-7419-4512.

Slonimskaya Elena M., Dr. Sci. (Med.), Professor, Faculty of Medicine, Department of Oncology, Saint-Petersburg State University, Saint-Petersburg. ORCID 0000-0003-4382-5697.

Litviakov Nikolai V., Dr. Sci. (Biology), Head of the Laboratory Oncovirology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk. ORCID 0000-0002-0714-8927.

(✉) **Ibragimova Marina K.,** e-mail: imk1805@yandex.ru.

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