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Transcriptome of Breast Tumors With Different Amplification Status of the Long Arm of Chromosome 8

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Abstract. *Background: Amplification of chromosome 8q with locus 8q24 is the most common copy number aberration, and is associated with tumour progression and chemoresistance. Materials and Methods: The study used paired samples of biopsy and surgical material from 60 patients with breast cancer. The amplification status of 8q was determined using a CytoScan HD Array microarray; complete transcriptomic analysis was performed using a Human Clariom S Assays microarray (Affymetrix, USA). Results: It was shown that in 65% of cases, amplification of 8q was preserved in the tumour after neoadjuvant chemotherapy (NAC). NAC significantly enhanced the heterogeneity of the transcriptome between tumours with and without amplification of 8q. Compared with a good response, a poor response to NAC also led to increased heterogeneity of the transcriptome of residual tumours. Eight differentially expressed genes of patients with different amplification status of 8q before and after NAC overlapped. Conclusion: Amplification of 8q leads to a significant shift in the level of transcription of a large number of genes after exposure to chemotherapy.*

According to the Progenetix database (www.progenetix.org), in terms of the frequency of occurrence in tumours of all localizations (177 types of tumours), amplification of the long arm of chromosome 8, particularly *8q*24, was the most common copy number aberration (CNA) and was found in more than 30% of all samples (1). Moreover, according to Catalogue of Somatic Mutations in Cancer, the most significant gene in this locus is the *MYC* proto-oncogene, which is involved in many signaling pathways (2). The frequency of propagation of amplification of *8q*, including the *MYC* gene, varies across different locations. It was shown that in lung cancer, the frequency of amplification of *8q* is notably high and amounted to 84.14% (69 out of 82 cases). Moreover, amplification of *MYC* on chromosome 8 was detected only in 32.9% of lung tumours (3). Additionally, with early gastric carcinoma, amplification of *8q*, as determined by microarray analysis, was observed in 77% (17/22) of cases, and the frequency of amplification of the *MYC* gene locus did not exceed 18.2% (4). Other authors using a significantly larger sample of patients with gastric cancer showed a greater than 30% amplification frequency of *8q*24, and amplification of the *MYC* gene was associated with adverse outcomes (5). The work of Gaelle Fromont and colleagues is interesting – they analysed a large sample of patients (n=242) with prostate cancer and determined the relationship of the status of amplification of the *8q*24 locus in the tumour tissue with the stage and course of the disease, and the probability of relapse after treatment. *MYC* amplification was observed in 29% of these cases and was closely associated with disease progression (p=0.001). *MYC* amplification status was also an independent predictor of relapse after prostatectomy (6).

At the same time, Letessier *et al*. conducted microarray studies using 547 breast tumour samples and showed that amplification of the *MYC* gene locus was present in only 6.1% of cases (7). Another study showed the frequency of amplification of the long arm of chromosome 8, including the *MYC* gene, to be 48.3% (29/60) in breast tumours (8). In the case of invasive lobular carcinoma of the mammary gland, the amplification frequency of *8q*24 of the *MYC* locus was 17% (24/70) (9). A recent study showed that the CNA frequency of the *MYC* gene locus in invasive ductal non-specific breast carcinoma, the primary histological type of breast cancer, was 54% (64/119). A high CNA frequency of *MYC* gene was associated with an adverse outcome, while with a good response to pre-surgery therapy, it resulted in elimination of clones with amplification of *8q*24 (10). This high prevalence of amplification of the long arm of

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chromosome 8 in tumours of various locations and in breast cancer, and the association of *8q*24 amplifications with an adverse prognosis and chemoresistance shows the importance of the copy number of this chromosome region in tumour progression and the relevance of research on this subject. In most cases, co-amplification of *8q* and expression are studied in terms of genes localized in the long arm of *8q*, and there are notably few studies on the transcriptome depending on the amplification status of *8q*. In addition, to date, we found no publications addressing the issue of transcriptomic change together with the amplification status of *8q* in the course of pre-surgery.

The aim of this work was to study changes in breast tumour transcriptome and the amplification status of the long arm of chromosome 8 (with locus *8q*24) according to changes in the process of pre-surgery chemotherapy.

Materials and Methods

Ethics approval and consent to participate. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was conducted with permission by the local Ethics Committee of the Cancer Research Institute Tomsk NRMC (Protocol 1 from January 14, 2013). All patients gave their informed consent. The experimental protocols were approved by institutional committee of Tomsk National Research Medical Center of the Russian Academy of Sciences (Protocol 3 from January 16, 2013).

Patients and treatment. The study included 60 patients with T1-4N0-2M0 breast cancer (stage IIA-IIIB) according to the eighth edition of the tumour, node, and metastasis classification system of the Union for International Cancer Control (30), of luminal B subtype, with a morphologically verified diagnosis, and were aged 22-68 years (average=46.2±0.4 years). All patients received 6-8 courses of systemic NAC based on anthracycline, anthracycline and taxane or taxotere in monotherapy mode. A physical examination was performed prior to NAC and before the surgery to determine the clinical response.

Primary breast lesions were visualized using mammography and ultrasound, and clinical and imaging responses were classified as follows: Complete clinical response (complete regression), partial regression, stabilization, and progression. An immunohistochemical study was performed to determine the molecular subtype of each tumour before treatment. The luminal B subtype of breast cancer was defined as oestrogen receptor (ER)-positive, progesterone receptor-negative or -positive, and Ki67 >30%, and all patients with luminal B subtype had human epidermal growth factor receptor 2-negative status. Patients underwent radiation therapy and/or hormone therapy after surgery. Hormone therapy was prescribed to all patients with the luminal B subtype. Radiation therapy was prescribed in the presence of lymphatic metastases (Table I).

The material for the study was samples of biopsy material before treatment paired with surgical material for each of the patients. The biopsy material of the tumour was taken prior to treatment using a pistol biopsy under ultrasound control.

Table I. *Clinical and morphological parameters of the examined patients with breast cancer.*

Parameter	Number of patients (%)
Age	
≤45 Years	24 (40.0%)
>45 Years	36 (60.0%)
Menstrual status	
Premenopausal	32 (53.3%)
Postmenopausal	28 (46.7%)
Histological type	
Invasive ductal carcinoma	50 (83.3%)
Invasive lobular carcinoma	2 (3.3%)
Medullary cancer	1 (1.7%)
Other type	7 (11.7%)
Tumor size	
T_1	6 (10.0%)
T_2	50 (83.3%)
T_3	3 (5.0%)
T_4	1 (1.7%)
Lymph node metastasis	
N_0	29 (48.3%)
N_1	24 (40.0%)
N_2	2 (3.3%)
N_3	5 (8.4%)
Molecular subtype	
Luminal B	60 (100%)
Response to NAC	
Progression	2 (3.3%)
Stabilization	15 (25.0%)
Partial regression	34 (56.7%)

NAC: Neoadjuvant chemotherapy. All patients received 6-8 courses of systemic NAC based on anthracycline, anthracycline and taxane or taxotere in monotherapy mode.

DNA and RNA isolation. DNA was isolated from these samples using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) in keeping with the manufacturer's instructions. RNA from surgical material after surgical treatment was isolated using RNeasy Plus Mini Kit (Qiagen) in keeping with the manufacturer's instructions.

Microarray analysis. The presence of amplifications in the long arm of chromosome 8 before and after NAC was determined using a CytoScan HD Array microarray (Affymetrix, Santa Clara, CA, USA). Gene expression was evaluated in 32 patients using a Human Clariom S Assays microarray (Affymetrix), and differentially expressed genes (DEGs) were determined depending on the presence of amplification of *8q*, changes in the process of NAC and the effect of NAC. Chromosome Analysis Suite 4.0 and Transcriptome Analysis Console 4.0 software (Affymetrix) were used to process the results of microchipping (bioinformatic analysis).

Results

The frequency of amplification of *8q* with region *8q*24 in the tumours of patients before treatment was 62% (37/60 cases). Out of 37 patients, amplification of *8q* in the residual tumour

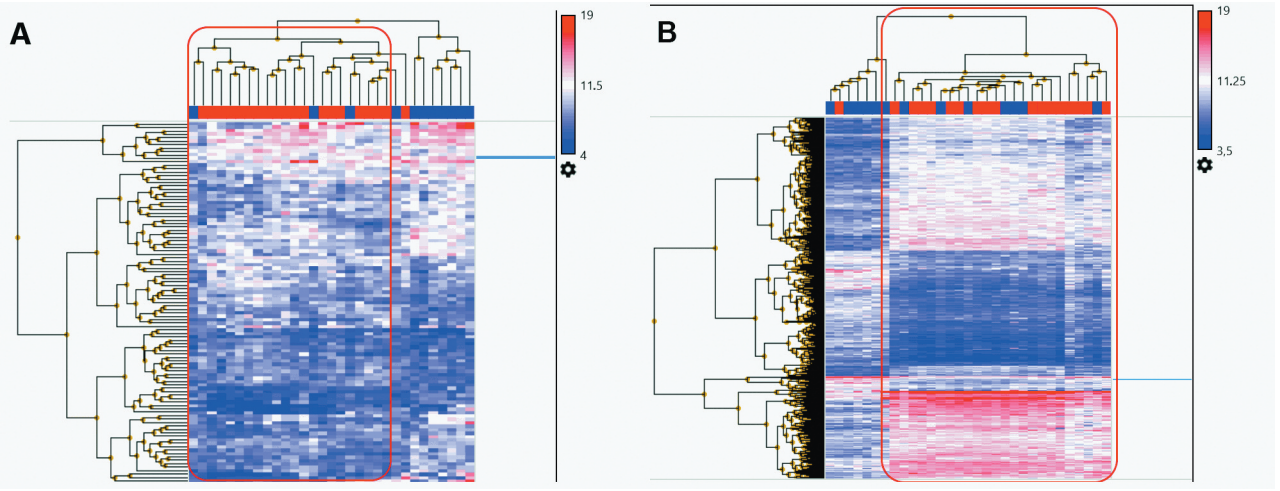

Figure 1. *Heat map of differentially expressed genes in the tumours of patients with breast cancer with (red) and without (blue) 8q amplification before treatment (A) and after neoadjuvant chemotherapy (B).*

after NAC was preserved in 24/37 patients (65%). In addition, in another three patients, amplification of *8q* in the tumour occurred *de novo* under the influence of pre-surgery therapy, which suggests the relative chemoresistance of tumour clones with amplification of *8q*.

In the tumour before treatment, the number of genes differentially expressed between patients with amplification of *8q* and those without amplification was 105 (41 up-regulated, 64 down-regulated) (Supplementary Table I, available at: https://docs.google.com/spreadsheets/d/1OI5ps4Kt0noyEm2puTTmCy9GqRYfaj34WQ524B8V-Pk/edit?usp=sharing). After NAC, the genes expressed in residual tumours of patients with and without amplification of *8q* differed significantly, with 2,174 DEGs (1,394 up-regulated, 780 down-regulated) (Figure 1). The top 10 DEG signalling pathways in the tumours of patients with breast cancer before treatment included meta-pathway biotransformation phases I and II; glucuronidation; podnet: protein–protein interaction in the podocyte; phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway; mitogen-activated protein kinase (MAPK) signalling pathway; head and neck squamous cell carcinoma; RNA polymerase III transcription; amino acid metabolism; pathways affected in adenoid cystic carcinoma; circadian rhythm-related.

The top 10 DEG signalling pathways in the tumours of patients with breast cancer after NAC included olfactory receptor activity; miR-targeted genes in lymphocytes; non-alcoholic fatty liver disease; cytoplasmic ribosomal proteins; miR-targeted genes in muscle cells; the vascular endothelial growth factor (VEGF)-A/VEGF receptor 2 signalling pathway; epidermal growth factor (EGF)/EGF receptor signalling pathway; nuclear receptor meta-pathway; MAPK signalling pathway; circadian rhythm-related genes. The common signalling pathways for patients before treatment and after NAC were the MAPK signalling pathway and circadian rhythm-related genes.

The construction of a Venn diagram showed that in patients with different amplification status of *8q* (with region *8q*24) before and after NAC, only eight DEGs overlapped (Table II).

It was further shown that with partial tumour regression, the number of DEGs in residual tumour after NAC was 879 (601 up-regulated, 278 down-regulated). During disease stabilization, the number of DEGs in residual tumour of patients after NAC was 1,321 (652 up-regulated, 669 down-regulated) (Figure 2). Upon disease stabilization, transcriptome heterogeneity between tumours with and without amplification of *8q* was enhanced. The top 10 DEGs in patients with stabilization of disease and partial regression, according to the amplification status of *8q*, are presented in Tables III and IV, respectively.

The construction of a Venn diagram showed that DEGs in patients were affected by partial regression and stabilization, with overlap in 145 genes (data are presented in Supplemental Table II, available at: https://docs.google.com/spreadsheets/d/1mbmaAzlNiZvTCQxHnOGpoGW2If-zRrkqJMWJGKOUZXk/edit?usp=sharing).

We have previously shown that genes whose functions are associated with the induction and maintenance of the stem state of cells – stemness genes – have a significant role in the mechanisms of metastasis of a breast tumour. Fifty-one such genes were selected from the databases, including the *MYC* gene located in *8q*24. It was shown that the amplification of stemness genes enabled tumour cells to acquire the ability to change from tumour non-stem cells to tumour stem cells and

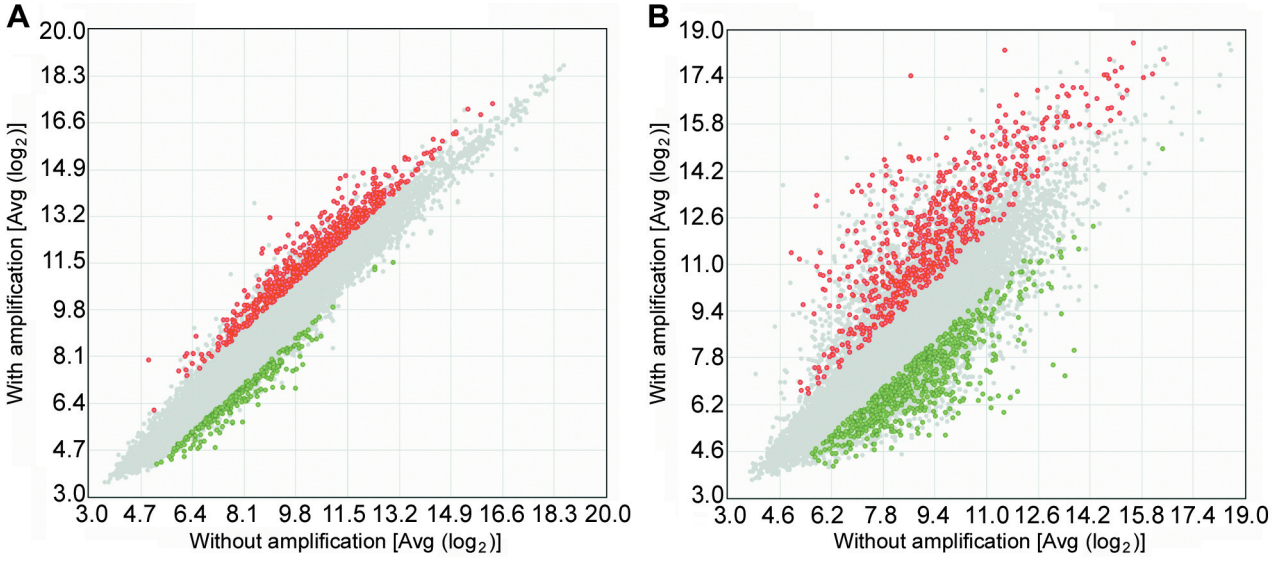

Figure 2. *The patterns of differentially expressed genes (red: up-regulated and green: down-regulated) in the residual tumour after neoadjuvant chemotherapy with and without amplification of 8q during partial regression (A) and during stabilization (B).*

to achieve metastasis. Increased expression of stemness genes was observed in a residual tumour in patients who subsequently developed metastases (11).

In the present study, according to the Venn diagram, 8/47 overlapping stemness genes [glycogen synthase kinase 3 beta (*GSK3B*), telomerase reverse transcriptase (*TERT*), bone morphogenetic protein 6 (*BMP6*), *MYC*, gata binding protein 3 (*GATA3*), NANOG homeobox (*NANOG*), SMAD family member 2 (*SMAD2*), and *SMAD4*] and DEGs were found between patients with and without amplification of *8q* after NAC (Figure 3). Moreover, in patients with amplification of *8q*, the expression of *GSK3B*, *MYC*, *GATA3*, *SMAD2* and *SMAD4* increased 2.1-3.8 times, and the expression of *TERT*, *BMP6*, and *NANOG* decreased 2.1-2.3 times. Thus, the amplification of the region of localization of *MYC* gene in a residual tumour resulted in increased expression of a complex of genes that are also involved in Wingless/Int (WNT) and transforming growth factor-β (TGFβ) signalling.

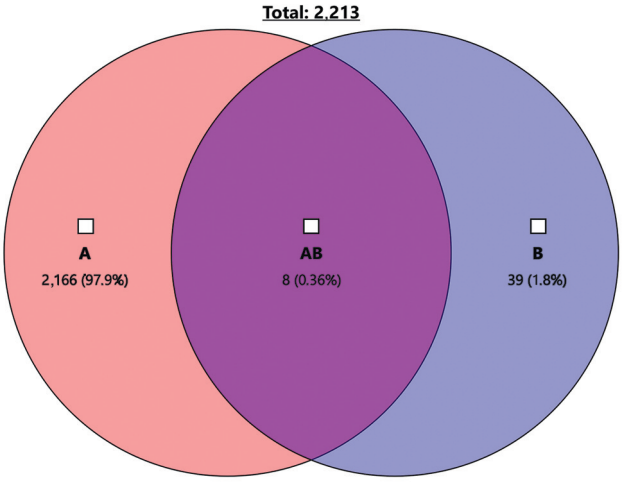

Figure 3. *Venn diagram showing overlapping differentially expressed genes in patients according to amplification of 8q after neoadjuvant chemotherapy and stemness genes. A: Genes differentially expressed after NAC (n=2,174); B: stemness genes (n=47).*

Discussion

According to the results of this study, carrying out NAC significantly enhances the heterogeneity of the transcriptome in tumours with amplification of *8q* compared with those without. At the same time, the number of up-regulated genes increased compared to that observed in patients before treatment and the number of down-regulated genes almost doubled. Figure 1B clearly shows that DEGs can be divided into three clusters; the first and third clusters were found to be overexpressed in patients with amplification of *8q* compared to those without amplification. The second cluster, on the contrary, was found to be hypoexpressed. Expression of ribosomal proteins increased especially significantly in patients with amplification of *8q* (at the beginning of cluster 3 in Figure 1B): *RPS21*, *RPS29*, *RPS30*, *RPS23*, *RPS28*, *RPL37A*, *RPS14*, *RPS8*, *RPL27*, *RPL35A*, *RPS20*, *RPL7A*, and *RPS12*, which may indicate a sharp increase in protein synthesis after exposure to NAC.

Table II. *Overlapping differentially expressed genes in patients with/without amplification of 8q before treatment and after neoadjuvant chemotherapy.*

Gene	Full title	Function	Localization
NBPF4	Neuroblastoma breakpoint family, member 4	This gene family consists of dozens of duplicated genes, mainly located on chromosome 1. Members of this family are characterized by tandemly repeating copies of DUF1220 protein domains. Changes in the number of gene copies in chromosome region *1q21.1* where most DUF1220 domains are located are involved in a number of developmental and neurogenesis diseases, such as autism, schizophrenia, congenital heart disease, neuroblastoma, congenital malformations of kidneys and urinary tract. Altered expression of some members of the gene family is associated with several types of cancer.	1p13.3
PI4KB	Phosphatidylinositol 4-kinase, catalytic, beta	The related pathways of this gene are the super pathway of inositol phosphate compounds and metabolism. Annotation of the gene ontology associated with this gene includes transferase activity, transfer of phosphorus-containing groups and the activity of 1-phosphatidylinositol-4-kinase. An important paralogue of this gene is *PI4KA*.	1q21.3
UGT2B11	UDP Glucuronosyltransferase family 2 member B11	This gene is a protein-encoding gene. Among its related pathways are drug metabolism- cytochrome P450 and metabolism of porphyrins and chlorophyll. Annotation of gene ontology related to this gene includes carbohydrate binding and glucuronosyl transferase activity. An important paralogue of this gene is *UGT2B28*.	4q13.2
UGT2B28	UDP Glucuronosyltransferase family 2 member B28	This gene encodes a member of the uridine diphosphoglucuronosyl transferase protein family. The encoded enzyme catalyses the transfer of glucuronic acid from uridine diphosphoglucuronic acid to a variety of substrates, including steroid hormones and fat-soluble drugs. This process is an intermediate step in steroid metabolism.	4q13.2
PLAT	Plasminogen activator, tissue	This gene encodes a tissue-type plasminogen activator and a secreted serine protease which converts plasminogen proenzyme into plasmin. It plays a role in cell migration and tissue remodelling.	8p11.21
MYBPC1	Myosin binding protein C, slow type	This gene encodes members of the family of myosin-binding protein C (myosin-associated proteins found in the transverse bridge region). The encoded protein is a slow isoform of the skeletal muscle of myosin-binding protein C and plays an important role in muscle contraction by attracting muscle-type creatine kinase to myosin strands. Mutations in this gene are associated with distal arthrogryposis of type I.	12q23.2
SETBP1	SET binding protein 1	This gene encodes the protein of the SET binding region. The encoded protein has been shown to bind the nuclear oncogene SET which is involved in DNA replication.	18*q*12.3
ZNF223	Zinc finger protein 223	This gene encodes a protein containing multiple zinc finger domains. The function of this protein has not been determined to date.	19q13.31

Of the hypoexpressed genes, 84/380 olfactory receptor activity genes in the zone of the middle gene cluster should be noted. Ligand activation of this signaling pathway has been found to inhibit the proliferation of prostate cancer cells (12), enhance apoptosis and inhibit the proliferation of non-small-cell lung cancer cell lines (13). At the same time, individual genes and proteins of this signaling pathway are tissue-specific tumour markers and promote tumour progression. For example, olfactory receptor family 7 subfamily C member 1 (*OR7C1)* was correlated with adverse prognosis in patients with colorectal cancer (14). Additionally, the expression of *OR2B6* was detected in breast carcinoma tissues: *OR2B6* transcripts were found in 73% of all breast cancer cell lines and in more than 80% of analysed breast cancer tissues (15). In our study, the expression of *OR2B6* was not different in patients with different amplification status of *8q*.

Table III. *The top 10 differentially expressed genes in patients with stabilization of disease according to the amplification status of 8q after treatment.*

ID	Gene symbol	Encoded protein	Expression (Ave. $\log_2$) *8q* Status		Fold change	*p*-Value	Cytoband
			Amplified	Not amplified			
1	*OR4F15*	Olfactory receptor family 4 subfamily F member 15	7.2	13.42	–74.36	0.013	15q26.3
2	*PROK2*	Prokineticin 2	8.06	13.71	–50.3	0.0369	3p13
3	*OR11H1*	Olfactory receptor family 11 subfamily H member 1	5.95	11.53	–48.01	0.0407	22q11.2
4	*OR4F17*	Olfactory receptor family 4 subfamily F member 17	7.55	13.05	–45.21	0.0282	19p13.3
5	*SSX2, SSX2B*	SSX family member 2; SSX family member 2B	6.44	11.75	–39.52	0.0338	Xp11.22
6	*CP*	Ceruloplasmin	6.67	11.95	–38.79	0.0262	3q23-q25
7	*CT45A3, CT45A4, CT45A5*	Cancer/testis antigen family 45 member A3, A4 and A5	6.1	11.14	–32.78	0.0183	Xq26.3
8	*USP17L23*	Ubiquitin specific peptidase 17-like family member 23	6.5	11.38	–29.4	0.0323	
9	*CT45A7, CT45A6*	Cancer/testis antigen family 45 member A7 and A6	6.4	11.11	–26.26	0.0191	Xq26.3
10	*OR1S2*	Olfactory receptor family 1 subfamily S member 2	5.15	9.72	–23.87	0.0358	11q12.1
10	*NDUFA2*	NADH:Ubiquinone oxidoreductase subunit A2	11.41	4.96	87.58	0.0283	5q31.2
9	*PRDX1*	Peroxiredoxin 1	14.37	7.9	88.72	0.0095	1p34.1
8	*TMED2*	Transmembrane P24 trafficking protein 2	14.05	7.47	95.69	0.0482	12q24.31
7	*SERF2*	Small EDRK-rich factor 2	13.51	6.84	101.86	0.0179	15q15.3
6	*MRPL42*	Mitochondrial ribosomal protein L42	13.71	7.01	103.95	0.0164	12q22
5	*SNRPD2*	Small nuclear ribonucleoprotein d2 polypeptide	14.6	7.84	108.16	0.0259	19q13.2
4	*RPS21*	Ribosomal protein S21	18.33	11.56	109.16	0.0122	20q13.3
3	*TMED10*	Transmembrane P24 trafficking protein 10	13	5.72	155.37	0.0266	14q24.3
2	*HIST1H2BG*	H2B Clustered histone 8	13.38	5.75	198.16	0.0065	6p22.2
1	*RPS29, RPL32P29*	Ribosomal protein S29; ribosomal protein L32 pseudogene 29	17.46	8.66	444.42	0.0105	14q; 14q21.3

In a study by Salhia *et al*., a complex genomic and epigenomic analysis of breast tumours with metastasis in the brain (n=23) was performed. Frequently amplified and overexpressed genes included ATPase family AAA domain-containing 2 (*ATAD2),* B-raf proto-oncogene, serine/threonine kinase *(BRAF),* derlin 1 *(DERL1)* and NIMA-related kinase 2 (*NEK2A)*. The authors concluded that the *ATAD2* and *DERL1* genes may play important roles in brain metastasis in breast cancer. At the same time, *ATAD2* is a transcriptional coactivator of oestrogen receptor 1 (*ESR1)* that is necessary to induce the expression of target genes of oestradiol, such as cyclin D1 (*CCND1), MYC*, and E2F transcription factor 1 (*E2F1)* (16). In our study, *ATAD1* and *DERL1* genes were overexpressed in tumours after NAC in patients with amplification.

Although the DEGs before and after NAC in patients with different amplification statuses of *8q* varied, there were also eight common DEGs, namely NBPF member 4 (*NBPF4),* phosphatidylinositol 4-kinase beta *(PI4KB),* UDP glucuronosyltransferase family 2 member B11 *(UGT2B11), UGT2B28,* plasminogen activator tissue type *(PLAT),* myosin binding protein C1 *(MYBPC1),* SET binding protein 1 *(SETBP1),* and zinc finger protein 223 *(ZNF223)*. A number of studies show the significance of these genes in tumour progression. The *NBPF4* gene can play the role of a potential biomarker in lung cancer and may be an oncogene (17, 18). Approximately 85/852 (10%) breast tumour samples showed amplification of the *PI4KB* gene (19). The *UGT2B11* gene, among other genes involved in lipid/fatty acid/steroid metabolism and lipid biosynthesis, showed differential expression in breast tumours compared to normal tissue and ER-negative cell lines, compared to ER-negative cell lines (American Type Culture Collection), as well as the *UGT2B28* gene (20, 21). The latter gene is also associated with the progression of prostate cancer (22). The *PLAT* gene

Table IV. *The top 10 differentially expressed genes in patients with partial regression of disease according to the amplification status of 8q after treatment.*

ID	Gene symbol	Encoded protein	Expression (Ave. $\log_2$) *8q* Status		Fold change	*p*-Value	Cytoband
			Amplified	Not amplified			
1	*LGI1*	Leucine-rich glioma inactivated 1	6.97	9.79	–7.1	0.0088	10q23.33
2	*PTPRR*	Protein tyrosine phosphatase receptor type R	6.98	9.37	–5.25	0.0016	12q15
3	*OR1S2*	Olfactory receptor family 1 subfamily S member 2	4.78	7.13	–5.1	0.0441	11q12.1
4	*DEFB107B; DEFB107A*	Defensin beta 107B and 107A	5.71	8.06	–5.07	0.0101	8p23.1
5	SLC28A3	Solute carrier family 28 member 3	7.83	10.04	–4.62	0.0202	9q21.32-q21.33
6	*OR4C12*	Olfactory receptor family 4 subfamily C member 12	6.36	8.55	–4.54	0.0268	11p11.12
7	*OR2M7*	Olfactory receptor family 2 subfamily M member 7	7.65	9.81	–4.46	0.0202	1q44
8	*PCP4*	Purkinje cell protein 4	7.58	9.7	–4.35	0.0386	21q22.2
9	*TSHB*	Thyroid-stimulating hormone subunit beta	5.67	7.73	–4.15	0.0111	1p13.2
10	*FAM177B*	Family with sequence similarity 177 member B	6.99	9.04	–4.13	0.0025	1q41
10	*FAM73A*	Family with sequence similarity 73, member A	11.97	9.15	7.05	0.0091	1p31.1
9	*PFN2*	Profilin 2	12.52	9.56	7.79	0.0243	3q25.1
8	*CRABP2*	Cellular retinoic acid-binding protein 2	14.21	11.2	8.1	0.0084	1q23.1
7	*ADIPOQ*	Adiponectin, C1Q and collagen domain-containing	7.99	4.95	8.26	0.0164	3q27.3
6	*FZD6*	Frizzled class receptor 6	12.93	9.85	8.43	0.0229	8q22.3-q23.1
5	*FIS1*	Fission, mitochondrial 1	11.86	8.69	9.01	0.0173	7q22.1
4	*CYB5A*	Cytochrome B5 type A	14.68	11.45	9.43	0.0311	18q23
3	*HIST1H2BM*	H2B Clustered histone 14	12.02	8.71	9.91	0.0385	6p22.1
2	*ANAPC11*	Anaphase-promoting complex subunit 11	14.58	11.27	9.92	0.0125	17q25.3
1	*FABP4*	Fatty acid-binding protein 4	13.16	8.94	18.64	0.0466	8q21

promoter was activated upon overexpression of paired related homeobox 2 (*PRRX2),* which enhanced migration and invasion and induced partial epithelial mesenchymal transition of MCF10A breast cells (23). Among other genes, *MYBPC1*, *PLAT*, and *SETBP1* showed low expression in a triple-negative breast tumour (n=163) compared with normal tissue (n=60) (24). On the other hand, inhibition of the expression of *SETBP*1 by microRNA led to a decrease in the proliferation and metastasis of triple-negative breast cancer cells (25). Sequencing of breast tumour DNA revealed nonsynonymous mutations in the *SETBP1* gene among other genes (26). *ZNF223* is among the top 40 most significant DEGs in dexamethasone-treated lymphoma cell samples compared to untreated cells (27).

An increase in the expression of the stemness genes *GSK3B, MYC, GATA3, SMAD4,* and *SMAD2* participating in WNT and TGFβ signalling was found in the residual tumour of patients with amplification of *8q*. The role of these two signalling systems in tumour progression is well-known. According to Shibue and Weinberg, TGFβ signalling plays a key role in the induction of epithelial–mesenchymal transition in tumour cells and their acquisition of the stem phenotype, which determines the invasive ability of tumours and the formation of secondary metastatic colonies (28). Wnt signalling determines the exit of tumour cells from replicative ageing after drug therapy. As shown by Milanovic and colleagues, after chemotherapy is ended, the activity of the WNT signalling pathway significantly increases in tumour cells, the number of stem tumour cells increases sharply, and tumour growth becomes even more active than before chemotherapy (29).

Conclusion

The study shows a large effect of amplification of the long arm of chromosome 8 on the tumour transcriptome in breast cancer, regardless of other molecular genetic features. Amplification of *8q* involving region *8q*24 leads to a significant change in the level of transcription of a large number of genes immediately after exposure to chemotherapy. For many of these genes, a role in tumour progression has been shown, which may be due to the known

association of amplification of *8q* with tumour progression, which has been shown in many *8q* locations.

Amplification of *8q* with the participation of region *8q*24, in which the *MYC* gene is localized, leads not only to an increase in the expression of this gene in the residual tumour but also to an increase in factors related to WNT and TGFβ signaling, which play a key role in the epithelial–mesenchymal transition and the exit of tumour cells from replicative ageing due to the effects of chemotherapy.

This leads to rapid tumor progression in patients with breast cancer with *MYC* gene amplification after adjuvant therapy (chemotherapy or hormonal). It makes sense to think about a significant increase in adjuvant chemotherapy timing for these patients. This will enable maintenance of disseminated tumor cells in a state of replicative aging for a long time and prevent the development of metastases.

Conflicts of Interest

The Authors declare that they have no conflicts of interests to report.

Authors' Contributions

Marina K. Ibragimova: Formal analysis, investigation, writing original manuscript. Matvey M. Tsyganov: Resources. Alina M. Pevzner: Resources. Nikolai V. Litviakov: Conceptualization, data curation, methodology, project administration, supervision, writing review and editing.

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