ASSOCIATION OF THE COMBINATION OF STEMNESS GENE AMPLIFICATIONS AND COPY NUMBER ABERRATIONS OF WNT-SIGNALING GENES IN BREAST TUMORS WITH METASTASIS

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Abstract

We studied the association between the presence of 2 or more stemness gene amplifications as well as copy number aberrations (CNAs) of WNT signaling genes in residual breast tumor and metastasis. WNT pathway genes associated with metastasis were identified. Material and Methods. The study included 30 patients with breast cancer, who had 2 or more stemness gene amplifications in the residual tumor after neoadjuvant chemotherapy. Fifteen of the thirty patients developed hematogenous metastases; they constituted a group with metastases, the remaining 15 patients entered the second group without metastases. The tumor DNA was examined using a CytoScanHD Array microarray (Affymetrix, USA). Results. By subtracting amplification and deletion frequencies in 852 cytobands between groups with metastases and without metastases, 21 cytobands were identified with the largest difference in deletion and amplification frequencies. They contain 19/150 of WNT genes (12 activators: SKP1, WNT8A, MAPK9, CCND3, FZD9, WNT8B, CCND1, PLCB2, PRKCB, FZD2, WNT3, WNT9B and 7 negative regulators: GSK3B, APC, CSNK2B, SFRP5, BTRC, TCF7L2, CSNK2A2). A point system was developed: when amplifying WNT-signaling activators or deletion of negative regulators, one point was added to the total score, and vice versa when deleting WNT-signaling activators or amplification of negative regulators, one point was taken from the total amount. It was shown that 93% (14/15) of patients with metastases had a total score higher than 0, while 93% (14/15) of patients without metastases had a total score of zero or less than zero. The differences between the groups were statistically significant according to the two-sided Fisher test with a high level of confidence probability (p=0.000003) and the log-rank test (p=0.00004) when assessing non-metastatic survival by the Kaplan-Mayer method. Conclusion Nineteen WNT signaling genes were identified. Copy number aberrations of these genes in combination with stemness gene amplifications in residual tumors were associated with metastasis. A new highly effective prognostic factor for breast cancer was identified.

Key words: breast cancer, WNT-pathway, metastasis, copy number aberration, residual tumor.
АССОЦИАЦИЯ СОЧЕТАНИЯ АБЕРРАЦИЙ ЧИСЛА КОПИЙ ГЕНОВ WNT-СИГНАЛИНГА И АМПЛИФИКАЦИЙ ГЕНОВ СТВОЛОВОСТИ В ОПУХОЛИ МОЛОЧНОЙ ЖЕЛЕЗЫ С МЕТАСТАЗИРОВАНИЕМ

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Аннотация
Введение. Изучена ассоциация с гематогенным метастазированием наличия 2 и более амплификаций генов стволовости и CNA (Copy Number Aberration) локусов генов WNT-сигнального пути в остаточной резидуальной опухоли молочной железы. Идентифицированы гены WNT-pathway, ассоциированные с метастазированием. Материал и методы. В исследование были включены 30 больных раком молочной железы, в резидуальной опухоли которых после неоадъювантной химиотерапии были 2 и более амплификации генов стволовости. У 15 из 30 больных развился гематогенный метастаз, они составили группу с метастазами, во вторую группу без метастазов вошли остальные 15 пациентов. ДНК опухоли была исследована при помощи микроматрицы CytoScanHd Array (Affymetrix, USA). Результаты. Путем вычитания частот амплификаций и делеций по 852 цитобендам между группами с метастазами и без метастазов был установлен 21 цитобенд с наибольшей разницей частот делеций и амплификаций. В них находятся 19 из 150 генов WNT (14 активаторов: SKP1, WNT8A, MAPK9, CCND3, FZD9, WNT8B, CCND1, PLCB2, PRKCB, FZD2, WNT3, WNT9B и 7 негативных регуляторов: GSK3B, APC, CSNK2B, SFRP5, BTRC, TCF7L2, CSNK2A2). Была разработана балльная система, при амплификации активаторов WNT-сигналинга или делеции негативных регуляторов к общей сумме баллов прибавлялся один балл, и наоборот, при делеции активаторов WNT-сигналинга или амплификации негативных регуляторов от общей суммы баллов отнимался один балл. Показано, что 93 % (14/15) больных с метастазами имеют суммарный балл больше 0, в то время как 93 % (14/15) больных без метастазов имеют суммарный балл, равный нулю или меньше нуля. Различия между группами статистически значимы по двустороннему критерию Фишера с высоким уровнем доверительной вероятности (р=0,000003) и по лог-ранговому тесту (р=0,00004) при оценке безметастатической выживаемости по методу Каплана – Майера. Заключение. Были идентифицированы 19 генов WNT-сигналинга, CNA которых в резидуальной опухоли, совместно с амплификацией генов стволовости ассоциированы с метастазированием и могут использоваться в качестве прогностического фактора.

Ключевые слова: рак молочной железы, WNT-сигналинг, метастазирование, аберрация числа копий, остаточная опухоль.
Laboratory and Experimental Studies

Sharply. Tumor growth became even more active than it was before chemo- and hormone therapy [2]. It remains unknown why in some cases WNT signaling pathways are activated, while in other cases they are not activated and some tumors do not progress.

Our working hypothesis is that activation of WNT signaling pathway in tumor cells is due to the presence of copy number aberration (CNA) of WNT signaling pathway genes. We believe that a tumor emerges from replicative aging caused by NAC and metastasizes if there are 2 or more amplifications of stemness genes (a necessary condition) and CNA genes of WNT signaling pathway. If there are only amplifications of stemness genes or CNA genes of WNT signaling pathway, a tumor does not metastasize.

In addition to emerging from replicative aging, WNT can directly induce stemness genes, thereby providing a significant increase in the number of tumor stem cells and the likelihood of metastasis. Some authors have shown that MYC, which is one of the stemness genes and is part of the Yamanaki cocktail, is directly connected to WNT signaling pathway and when WNT is attached to Frizzled receptors, expression of markers of epithelial-mesenchymal transition and stemness of SNAI2, MYC and others is activated. Canonical WNT signaling caused by LRP6 Cyclin Y/CDK14 priming helps stabilize β-catenin-independent proteins, block polyphosphorylation and polyubiquitination of target proteins, including c-Myc [3, 4]. Activation of β-catenin in WNT signaling pathway also stimulates hyperexpression of MYC [5].

In accordance with the working hypothesis, we identified WNT pathway genes, whose CNA together with the amplification of stemness genes, were associated with metastasis.

Material and Methods

Between 2009 and 2014, 30 patients with IIbIIIa (T2–3N0–1M0) luminal B breast cancer were treated at Cancer Research Institute of Tomsk NRMC (Tomsk, Russia). The age of the patients ranged from 35 to 62 years (average age 52.4 ± 0.5). All procedures followed the Helsinki Declaration (1964, amended in 1975 and 1983). This study was approved by the institutional review board, and all patients signed an informed consent for voluntary participation (tabl. 1). All patients received 6 to 8 cycles of systemic neoadjuvant chemotherapy with the AC (Adriamycin, and Cyclophosphamide) or AT/ACT (Adriamycin and Taxotere or Adriamycin, Cyclophosphamide and Taxotere), or Taxotere. Clinical and imaging responses were categorized according to WHO and International Union Against Cancer criteria [7]. A partial response (PR) was determined as a tumor reduction >50% and stable disease (SD) as a tumor reduction <50% or a tumor size increase of <25% and progressive disease (PD) as a tumor size increase of >25%. Physical examination was performed before NAC and before surgery to determine the clinical response. Surgery (radical resection, sectoral resection or mastectomy) was performed within three to four weeks after the last administration of chemotherapy in responsive patients. After surgery hormonal therapy was given.

DNA was extracted from fresh samples of post-NAC residual tumor tissues using the QIAlamp mini Kit (Qiagen, Germany #51304).

Microarray analysis. To study CNAs of breast tumor, microarray analysis was performed using high density microarray platform Affymetrix (USA) CytoScan™ HD Array, (http://www.affymetrix.com/esearch/search.jsp?pd=prod520004&N=4294967292). Procedures of sample preparation, hybridization and scanning were performed in accordance with the manufacturer’s protocol using the system Affymetrix GeneChip® Scanner 3000 7G (Affymetrix, USA). The Chromosome Analysis Suite 4.0 software (Affymetrix, USA), which was specifically devised for analyzing microarray results from the CytoScan™ HD Array, was used. Unbalanced chromosomal aberrations (deletions and amplifications, or Loss and Gain) were detected in all chromosomal regions.

Bioinformatics Methods. For each of 852 cytobands of patient groups with 2 or more amplifications of stemness genes with metastases and without metastases, the frequency of amplifications and deletions was determined and a histogram was constructed for each group. Afterwards, these histograms were combined. Thus, cytobands were calculated with the greatest difference in amplification and deletion frequencies. These data are shown in Figure 1. Using the KEGG GSEA database (http://software.broadinstitute.org/gsea/msigdb/cards/KEGG_WNT_SIGNALING_PATHWAY), 150 human WNT signaling genes were selected which are presented in Table 2. Using the GeneCards database (https://www.genecards.org/) the localization of each gene was determined. We found genes that were localized in cytobands with the highest frequency of amplifications and deletions according to the data presented in Fig. 1. These genes are highlighted in Table 2 in different colors. At the next stage, each of the selected genes was annotated using several databases: Reactome (https://reactome.org), UniPlot (https://www.uniprot.org), GeneCards (https://www.genecards.org/), OMIM (https://omim.org/), Wnt signaling pathway Gene Ontology Term (http://www.informatics.jax.org/vocab/gene_ontology/GO:0016055), KEGG PATHWAY: Wnt signaling pathway – Homo sapiens (human) (https://www.genome.jp.kegg/ pathway/hsa/hsa 04310.html) and the role of the gene in the regulation of WNT-pathway was determined. Also, articles were used for annotation.

Statistical analysis. A two-sided p-value was calculated using Fisher’s exact test http://vassarstats.net/odds2x2.html. Metastasis-free survival was calculated using the Kaplan-Meier method, and differences among patient groups were evaluated using the log-rank test.
Results

By subtracting the frequencies of cytoband amplifications and deletions in residual tumors of patients with 2 or more amplifications of stemness genes between groups with metastases (patients B1, G1, D1, E1, K1, K2, K7, L1, R1, S1, S2, S3, Ch1, Ch3, Yu1) and without metastases (patients D3, J1, J2, K6, L2, L3, M4, N1, P1, P3, P2, S7, S10, Ch2, S7, S10, Ch2, Sh1), we can see differences in the clinicopathological parameters of breast cancer patients and stemness gene amplifications in residual tumors.

### Table 1

| Пациенты/ Patients | Амплификации локусов генов стволовости в резидуальной опухоли/Stemness genes loci amplifications in residual tumor | T | N | Ответ на НАХТ/ NAC response % | Metastasis/Metastasis-free survival, month |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------|---|---|-------------------------------|------------------------------------------|
| B1 5p16p            | 2 1 74 PR Да/Yes                                                   | 25 |
| G1 3q5p8q           | 2 0 7 SD Да/Yes                                                   | 62 |
| D1 5p8q4q10q        | 2 1 86 PR Да/Yes                                                   | 19 |
| E1 3q16p18q         | 2 2 -66 PD Да/Yes                                                   | 47 |
| K1 7q8q             | 2 1 75 PR Да/Yes                                                   | 28 |
| K2 8q12p16p         | 2 0 0 SD Да/Yes                                                   | 105 |
| K7 3p6p8q9q13q      | 2 1 80 PR Да/Yes                                                   | 12 |
| L1 5p8q10p          | 2 3 28 SD Да/Yes                                                   | 10 |
| R1 5p7q9p10p        | 3 2 21 SD Да/Yes                                                   | 12 |
| S1 5p7q16p18q19p    | 2 2 45 SD Да/Yes                                                   | 11 |
| S2 5p6q9p10p18q19p  | 2 1 -5 SD Да/Yes                                                   | 20 |
| S3 5p6q7q8q13q19p   | 3 3 -35 PD Да/Yes                                                   | 17 |
| Ch1 5p7q10q8q16p    | 2 0 42 SD Да/Yes                                                   | 23 |
| Ch3 5p8q16q19p      | 3 3 60 PR Да/Yes                                                   | 21 |
| Yu1 6p8q13q         | 2 3 59 PR Да/Yes                                                   | 43 |

Примечание: PR – частичная регрессия, SD – стабилизация, PD – прогрессирование; все пациентки имели люминальный В молекулярный подтип РМЖ. Люминальный В подтип определялся как позитивный по ER и PR статусу и Ki67 > 30 %. ER+ – экспрессия экстрогеновых рецепторов больше 0, PR+ – экспрессия прогестероновых рецепторов больше 0. HER2 тестировали в соответствии с рекомендациями American Society of Clinical Oncology/College of American Pathologists Guideline 2007 Recommendation [6].

Note: PR – partial response, SD – stable disease, PD – progressive disease; All patients had a luminal B subtype. Luminal B subtype was determined when positive ER and PR status and Ki67 > 30 % were observed. ER+ expression of estrogen receptors more than 0, PR+ expression of progesterone receptors more than 0. HER2 testing is performed in accordance with American Society of Clinical Oncology/College of American Pathologists Guideline 2007 Recommendation [6].
### Table 2/Table 2

| Гены/Genes | Локализация/Cytoband | Наличие метастазов/Yes metastasis | Отсутствие метастазов/No metastasis | Функция/Function |
|---|---|---|---|---|
| GSK3B | 3q13.33 | Del 13% | Amp 33% | Один из ключевых негативных регуляторов канонического WNT-пути/One of the key negative regulators of canonical WNT-pathway |
| APC | 5q22.2 | Amp 20% | Del 33% | Негативный регулятор канонического WNT-пути/Negative regulator of canonical WNT-pathway |
| PPP2CA | 5q31.1 | Amp 20% | Del 20% | Позитивный регулятор канонического WNT-пути/Positive regulator of WNT-pathway |
| SKP1 | 5q31.1 | Amp 20% | Del 20% | Участвует в трансдукции сигнала WNT-пути, активатор/Participates in WNT-pathway signal transduction, activator |
| TCF7 | 5q31.1 | Amp 20% | Del 20% | Негативный регулятор канонического WNT-пути/Negative regulator of canonical WNT-pathway |
| WNT8A | 5q31.2 | Amp 20% | Del 20% | Активатор WNT-пути/WNT-pathway activator |
| MAPK9 | 5q35.3 | Amp 20% | Del 0% | Активатор TP53/Activator of TP53 |
| CSNK2B | 6p21.33 | Del 0% | Amp 20% | Позитивный регулятор канонического WNT-пути/Positive regulator of WNT-pathway |
| PPARD | 6p21.31 | Del 0% | Amp 20% | Позитивный регулятор канонического WNT-пути/Positive regulator of WNT-pathway |
| PPP2R5D | 6p21.1 | Amp 0% | Del 27% | Позитивный регулятор канонического WNT-пути/Positive regulator of WNT-pathway |
| CCND3 | 6p21.1 | Amp 0% | Del 27% | Активация пролиферации через WNT-пути/Activation of proliferation via WNT-pathway |
| FZD9 | 7q11.23 | Amp 20% | Del 0% | Активатор WNT-пути/WNT-pathway activator |
| CUL1 | 7q36.1 | Amp 20% | Del 0% | Позитивный регулятор канонического WNT-пути/Positive regulator of WNT-pathway |
| FRAT1 | 10q24.1 | Del 27% | Amp 7% | Позитивный регулятор канонического WNT-пути/Positive regulator of WNT-pathway |
| FRAT2 | 10q24.1 | Del 27% | Amp 7% | Позитивный регулятор канонического WNT-пути/Positive regulator of WNT-pathway |
| SFRP5 | 10q24.2 | Del 27% | Amp 0% | Негативный регулятор канонического WNT-пути/Negative regulator of canonical WNT-pathway |
| WNT8B | 10q24.31 | Del 27% | Amp 7% | Активатор WNT-пути/WNT-pathway activator |
| BTRC | 10q24.32 | Del 27% | Amp 7% | Негативный регулятор канонического WNT-пути/Negative regulator of canonical WNT-pathway |
| TCF7L2 | 10q25.2-q25.3 | Del 27% | Amp 0% | Негативный регулятор канонического WNT-пути/Negative regulator of canonical WNT-pathway |
| CCND1 | 11q13.3 | Amp 33% | Del 7% | Активация пролиферации через WNT-пути/Activation of proliferation via WNT-pathway |
| CHP1 | 15q15.1 | Amp 7% | Del 33% | Ингибитор NFAT неканонического WNT-пути/NFAT inhibitor of noncanonical WNT-pathway |
| PLCB2 | 15q15.1 | Amp 7% | Del 33% | Активатор WNT-пути/WNT-pathway activator |
| CSNK2A2 | 16q21 | Del 27% | Amp 7% | Активатор TP53/Activator of TP53 |
| PRKCB | 16p12.2-p12.1 | Amp 20% | Del 7% | Активатор WNT-пути/WNT-pathway activator |
| FZD2 | 17q21.31 | Amp 27% | Del 27% | Активатор WNT-пути/WNT-pathway activator |
| WNT3 | 17q21.31-q21.32 | Amp 27% | Del 20% | Активатор WNT-пути/WNT-pathway activator |
| WNT9B | 17q21.32 | Amp 27% | Del 20% | Активатор WNT-пути/WNT-pathway activator |
WNT signaling genes were localized: difference in deletion and amplification frequencies, 27 metastases, and the function of these genes.

In total, in the chromosomal regions with the largest difference in deletion and amplification frequencies, 27 WNT signaling genes were localized: GSK3B, APC, PPP2CA, SKP1, TCF7, WNT8A, MAPK9, CSNK2B, PPP2RD, CGG2, CCND3, FZD9, CUL1, FRAT1, FRAT2, SFRP5, WNT8B, BTRC, TCF7L2, CCND1, CHP1, PLCB2, CSNK2A2, PRKCB, FZD2, WNT3, WNT9B (tab. 2).

We divided all identified WNT-signaling genes into three large groups. The first group included activators of WNT-signaling (illic); it was composed of receptor ligands, some transcription factors, secondary messengers and transcription factors that play a key role in the work of the WNT-pathway. Also, this group included two cyclins D3 and D1, which trigger the cell cycle via WNT-signaling and are the endpoint of the WNT signaling pathway. In total, this group included 12 genes: SKP1, WNT8A, MAPK9, CCND3, FZD9, WNT8B, CCND1, PLCB2, PRKCB, FZD2, WNT3, WNT9B. Activation of WNT signaling should be significantly facilitated by deletions of chromosomal regions of localization of these genes and substantially hindered by deletions. The second group of genes included genes whose products, according to OMIM and GeneCards, negatively regulate the WNT signaling pathway (green). This group included 7 genes: GSK3B, APC, CSNK2B, SFRP5, BTRC, TCF7L2, CSNK2A2. Activation of WNT signaling should be significantly facilitated by deletions of chromosomal regions of these genes localization and significantly hindered by their amplifications. The third group was composed of positive regulators of canonical and noncanonical WNT signaling pathways (PPP2CA, TCF7, PPP2RD, CUL1, TCP41, FRAT1, FRAT2). It included some transcription factors, secondary messengers, kinases. They can exert a noticeable but not as critical effect as activators on the WNT-signaling pathway, while the activity of the products of these genes can either be suppressed by other factors, or they belong to the noncanonical pathway, or they regulate the manifestations of WNT signaling little associated with proliferation, migration, adhesion and stemming, i.e. those mechanisms that are necessary for metastasis. CNA regions of localization of these genes were excluded from further analysis due to the high pleiotropy of mutual influences and low significance for metastasis mechanisms. CHP1 belong to the noncanonical WNT-signaling pathway.
After such preliminary bioinformatics selection of WNT-signaling genes, the relationship with metastasis was assessed only for the first (lilac) and second (green) gene groups (tabl. 2). In accordance with the working hypothesis, metastasis should be facilitated by amplification of gene loci of the first group and deletion of loci of genes of the second group, while inhibition of metastasis should be done by deletion of gene loci of the first group and amplification of loci of genes of the second group. In accordance with this formulation, a point system was developed. One point was added to the total score when amplifying WNT-signaling activators or deletion of negative regulators, and vice versa, when deleting WNT-signaling activators or amplification of negative regulators, one point was taken from the total amount. The distribution of amplifications and deletions at all loci of the genes of the first and second groups and the total score for all 30 patients studied are presented in Fig. 2. As seen in Fig. 2, 93 % (14/15) of patients with metastases have a total score greater than 0, while 93 % (14/15) of patients without metastases have a total score of zero or less than zero. The differences between the groups are statistically significant according to the two-sided Fisher test with a high level of confidence probability (p=0.000003) and the log-rank test (p=0.000004) when assessing metastasis-free survival by the Kaplan-Mayer method (fig. 3).

Thus, our data indicate that CNA genes of WNT-signaling are associated with metastasis and the prognostic value of CNA genes of WNT-signaling in residual tumors is highly predictive, which confirms our working hypothesis that CNA genes of WNT signaling are involved in the implementation of the metastasis process. Amplifications of stemness genes give tumor cells the ability to do stem transition [1], amplifications of activators SKP1, WNT8A, MAPK9, CCND3, FZD9, WNT8B, CCND1, PLCB2, PRKCB, FZD2, WNT3, WNT9B and / or deletions of negative regulators of WNT signaling genes: GSK3B, APC, CSNK2B, SFRP5, BTRC, TCF7L2, CSNK2A2, contribute to the release of tumor cells from replicative aging and dormant state, and activation of stem transition.

Discussion

The association of the combination of stemness gene amplifications and WNT-signaling genes in residual tumors with hematogenous metastasis was studied. Based on the microarray study and using bioinformatics methods, the most important WNT-signaling genes were identified for 12 WNT signaling activator genes and 7 negative regulator genes, amplification and deletion (respectively) of which were associated with metastasis (according to the Fisher’s two-sided criterion, p=0.000003 and the log-rank test, p=0.000004, when evaluated by the Kaplan–Mayer method).

According to modern recommendations, adjuvant chemotherapy (ACH) is given to patients, who have not previously received 6–8 cycles of NACH. Current methods for predicting the risk of relapse (in particular, the Oncotype DX platform), which show the need for ACT are often inefficient, and even if they are used, the majority of patients (more than 40–50 % of patients) receive postoperative chemotherapy [8], while according to our data, only 25 % of patients need adjuvant chemotherapy. These are patients with 2 or more amplifications of stemness genes and a positive total CNA score of WNT signaling genes. Our new prognostic factor is currently one of the most highly effective for breast cancer.

Some of the WNT signaling genes that we have identified are considered in the modern literature in terms of metastasis mechanisms. Gene FZD9 gene

![Rис. 1. Частота опухолевых CNA (амплификации и делеции) у пациенток с метастазами и без них](image-url)
### Распределение амплификаций (Gain) и делеций (Loss) по всем локусам генов активаторов WNT (red) и негативных регуляторов (green) и суммарный балл по 30 обследованным больным.

**Рис. 2.**

| Локусы | Positive regulation | WNT-pathway activators (ligands, receptors, messengers) | Negative regulation | Negative regulator of WNT-pathway | Score |
|--------|---------------------|--------------------------------------------------------|----------------------|-----------------------------------|-------|
|        | SKP1, PPP2CA, TCP7 | WNT8A, MAPK11, CCND3, PPP2R5Q, FZD9                  | GSK3B, APC, CNK1       | ESR1, BRC, TCF7L2, CSNK2A2          |       |
|        | 5q31.1, 5q31.2, 5q35.3 | 5q21.1, 7q11.23, 10q24.31, 11q13.3, 15q15.1, 10q12.13, 17q12.1, 17q12.3 | 10q24.3, 16p12.1, 17q12.1, 17q21.32 | 3q13.3, 5q22.2, 6p21.13, 6q24.2, 10q24.32, 10q25.2, 16q21 |       |
| Yes metastasis | | | | | |
| B1 | 1 | n n n n n | n n | Loss | Gain | n n n n n | Gain | 3 n n n | Loss | Loss | n | 4 |
| G1 | 4 | Gain | Gain | Gain | n n | Gain | n n | n | Loss | Gain | n | 3 n n | n | Loss | Loss | Loss | n | 7 |
| D1 | 2 | n n n n n | n n | n n | Gain | n n | n n | n | n | Loss | 1 | n n n | n | Loss | Loss | Loss | n | 3 |
| E1 | 0 | n n n n n | n n | n n | Gain | n n | n n | n | n | Loss | 1 | n n n | n | Loss | Loss | Loss | n | 5 |
| K1 | 3 | n n n n n | Gain | n n | n n | Gain | n n | n | Gain | 1 | n n n | n | Loss | Loss | Loss | n | 4 |
| K2 | 0 | n n n n n | n n | n n | Loss | Gain | n n | n | n | Loss | 1 | n n n | n | Loss | Loss | Loss | n | 1 |
| K7 | 6 | Gain | Gain | Gain | n n | n n | Gain | n n | n | n | Gain | n | n | Gain | Loss | 5 |
| L1 | -1 | n n | Loss | Loss | n n | Loss | n n | n | Gain | n | 5 | n n | Loss | Loss | Loss | Loss | 4 |
| R1 | -2 | n n | n n | Loss | Gain | n n | Gain | n n | Loss | Loss | 3 | Lose | n n | Loss | n | Loss | 1 |
| S1 | -1 | Gain | Gain | n n | Gain | n n | Gain | n n | Loss | Loss | 2 | Loss | n n | Loss | Loss | n | 4 |
| S2 | -4 | Loss | Loss | Loss | Gain | n n | Loss | n n | Loss | Loss | 2 | Loss | n n | Loss | Loss | n | 4 |
| S3 | 3 | n n n n n | n n | Gain | n n | n n | Gain | n n | Gain | Gain | 4 | n n n | n n | Loss | Loss | Loss | 5 |
| Ch1 | 8 | Gain | Gain | Gain | n n | Gain | n n | Gain | Gain | Gain | 4 | n n n | n n | Loss | Loss | Loss | 5 |
| Ch3 | 3 | n n n n n | Loss | Loss | n n | Loss | n n | n n | n | Loss | 4 | n n n | n n | Loss | Loss | n | 4 |
| Yu1 | -3 | n n n n n | Loss | Loss | n n | Loss | n n | n n | n | Loss | 4 | n n n | n n | Loss | Loss | n | 4 |
| No metastasis | | | | | | |
| D3 | -3 | Loss | Loss | Gain | Loss | n n | Gain | n n | n | Loss | 2 | n n | Loss | n n | n | n | -4 |
| J1 | 0 | n n n n n | n n | n n | Gain | n n | n n | n | n | Loss | 1 | n n n | n n | Loss | n n | n | 0 |
| J2 | 3 | n n n n n | Gain | n n | n n | Gain | n n | n n | n | Loss | 1 | n n n | n n | Loss | n n | n | 0 |
| J6 | -2 | n n n n Loss | n n n n | Loss | n | n | Loss | n n | Gain | 1 | n n | Loss | n n | n | n | -4 |
| J2 | -1 | n n | n n | Loss | n n | n n | n n | n | n | Loss | 0 | n n | n n | n n | n | n | -4 |
| L3 | -1 | n n n n n | n n n n n | n n n n | Gain | n | n | n | n | n | n | Loss | n n | n n | n | n | -1 |
| M4 | 2 | Gain | Gain | n n | Loss | n n | n n | n | n | Gain | 0 | n n | n n | n | n | Gain | 0 |
| N1 | 5 | n n | Gain | Gain | n n | Loss | Gain | Gain | n n | Gain | 0 | Gain | n | Gain | Gain | n | n | n | 1 |
| P1 | -1 | Loss | Loss | Gain | n n | n n | Loss | Gain | n n | n n | -1 | Gain | n n | Gain | Gain | n | n | n | 2 |
| P3 | -3 | n n | n n | Loss | n n | n n | n n | n n | n n | Loss | -2 | Gain | n | Gain | Gain | n | n | n | 2 |
| P2 | -2 | n n | n n | Loss | n n | n n | n n | n n | n n | Loss | 0 | n n | n n | n n | n | n | n | 2 |
| S7 | -2 | Loss | Loss | n n | n n | n n | n n | n n | n n | Loss | 0 | n n | n n | n n | n | n | n | 2 |
| S10 | -5 | n n | n n | n n | Loss | n n | n n | n n | n n | Loss | 1 | n n | n n | n n | n | n | n | 1 |
| Ch2 | -4 | n n | n n | n n | Loss | n n | n n | n n | n n | Loss | 3 | n n | n n | n n | n | n | n | -1 |
| Sh1 | -5 | Loss | Loss | n n | Gain | n n | n n | n n | n n | Loss | 2 | n n | n n | n n | n | n | n | -3 |

**Примечание:** Серые ячейки с суммарным баллом > 0; n — нормальное состояние локуса

**Note:** Gray cell with a total score > 0; n is the normal state of the locus.
is activated after exposure of breast tumor cells to chemotherapy and is involved in the induction of the stem phenotype in these cells [9]. Amplification of gene CCND1 is associated with a poor outcome in breast cancer [10]. Knockdown of CCND1 inhibits mammosphere formation of breast cancer cells [11]. MiR-4779 inhibits CCND3 in colon cancer cells, causing cell cycle arrest and apoptosis [12]. Inhibition of SKP1 leads to mitotic arrest of lung cancer cells [13]. Inhibitors of other activators of WNT signaling genes are being developed: PPP2CA [14], WNT3 [15] and others. For FZD2, on the contrary, it was shown that its hyperexpression was accompanied by suppression of metastasis of salivary adenoid cystic carcinomas [16]. Previous studies have shown that during EMT and metastasis, Wnt5α/b ligand and/or its cognate receptor Fzd2 are generally overexpressed in colon, lung, colon, liver, and the gastric tract [17–19]. The WNT7A gene is associated with an unfavorable prognosis in patients with breast cancer treated with NAC [20].

As for negative regulators, there is much less information. The SNP of BTRC rs61873997 G>A gene was associated with the prognosis for skin cancer, and the presence of the wild allele had an unfavorable effect with HR=0.61 (0.46–0.80), p=0.00031) [21]. Suppression of negative WNT regulators does not always lead to inhibition of metastasis. Thus, it was shown that pharmacological inhibition of GSK3β activity stabilized the TRAF6 protein, promoted CTNNB1 degradation, and effectively suppressed EMT and CRC metastasis [22]. On the other hand, tamoxifen treatment of breast cancer cells increases the phosphorylation of GSK3β and, therefore, its activity [23]. Low expression of SFRP5 in human pancreatic ductal adenocarcinoma (PDAC) was a poor prognostic factor for human PDAC [24]. Epigenetic inactivation of the SFRP5 gene in human breast cancer is associated with unfavorable prognosis [25]. The APC gene is a known tumor suppressor gene and in breast cancer its deletion is associated with resistance to doxorubicin and cisplatin [26, 27].

The indirect effect of negative regulators should also not be neglected. It is well known that TP53 and CDKN2A genes are considered the main barrier to efficient conversion of normal cells into induced pluripotent stem cells. They inhibit spontaneous transformation of differentiated cells into pluripotent ones [28]. Some negative regulators are able to activate TP53 [3]. Apparently, deletions of these genes lead to a decrease in TP53 activity and, as a result, thresholds for transition of differentiated tumor cells to stem cells are reduced. This may be one of the explanations of the effect observed by Milanovic [2] which consists in a sharp increase in the number of tumor stem cells upon activation of WNT signaling after cancellation of chemo and hormonal therapy.

Conclusion
The results of the study confirm the working hypothesis that CNAs of WNT signaling genes are involved in the metastatic process. Nineteen WNT signaling genes were identified. Copy number aberrations of these genes in combination with stemness gene amplifications in residual tumors were found to be associated with metastasis. A new highly effective prognostic factor for breast cancer was developed.
growth by inducing apoptosis and cell cycle arrest through direct target-
neanu-Petric R., Balacescu O., Berindan-Neagoe I
Литвяков Николай Васильевич,
Diseases. 2020. doi: 10.1016/j.gendis.2020.01.015.
CCND1 in enhancing breast cancer stemness and metastasis. Genes &
Taftaf R., Wray B., Liu H
2006 Dec; 15(6): 718–27. doi: 10.1016/j.breast.2006.02.005.
ITGA2 promotes expression of ACLY and
Fang S., Lee J.E., Thompson A.M
Dahl E. 
Woo J., Kim J.H., Kwon H.
Stefanski C.D., Prosperi J.R.
Zhou W., Tian M., Hu J., Li, He Y. SFRP5 as a prognostic biomarker for patients with pancreatic ductal adenocarcinoma. Int J Clin Exp Pathol. 2016; 9(3): 3442–3447.

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