Original Paper

A Minimal lncRNA-mRNA Signature Predicts Sensitivity to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer

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Key Words

Triple-negative breast cancer • Neoadjuvant chemotherapy • Response • Gene signature • lncRNA

Abstract

Background/Aims: Triple-negative breast cancer (TNBC) is a highly aggressive malignancy that responds in a diverse manner to neoadjuvant chemotherapy (NAC). This study was aimed to uncover an RNA signature in TNBC patients which predicts pathological complete responses (pCR) to NAC by analyzing long noncoding RNA (lncRNA) and coding gene expression.

Methods: Microarray datasets from 26 TNBC patients receiving NAC including ten patients showing pCR were obtained from the Gene Expression Omnibus database. **Results:** A total of 172 coding genes and 84 lncRNAs were differentially expressed between patients achieving pCR and those who did not. Filtering based on the predictive efficacy of response to NAC using receiver operator characteristic curve (ROC) and area under the curve (AUC) shortlisted 23 lncRNAs and 15 coding genes from consideration. Finally, a response score consisting of 1 lncRNA and 2 coding genes was developed: response score = $2.595 \times \text{BPESC1} - 1.09 \times \text{WDR72} - 1.428 \times \text{GADD45A} - 0.731$. The response score had good predictive performance (AUC=0.931, $p < 0.01$) and at the cut-off of 0.545, the response score had sensitivity and specificity of 0.8 and 0.9, respectively. **Conclusion:** We propose a simple gene expression signature of only three RNA species could be employed clinically to predict pCR in TNBC patients receiving NAC.

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Introduction

Breast cancer is one of the most common types of malignant cancer worldwide and leads to about half a million deaths every year [1, 2]. Neoadjuvant chemotherapy (NAC) in patients with advanced disease can enhance surgical opportunities, particularly making breast-conserving surgery more feasible in patients presenting with large tumors [2]. Moreover, patients demonstrating a pathological complete response (pCR) to NAC have better prognosis than those with partial responses or progressive disease [3-5]. NAC has been widely used for these reasons but nevertheless pCR rates for NAC are low. The National Surgical Adjuvant Breast and Bowel Project report the pCR rate as 40-50% [6] while others suggest the rate may be even lower [7]. Thus, it is important to develop predictive markers for pCR to optimize the use of NAC.

Triple-negative breast cancer (TNBC) represents about 10%-20% of all breast cancer cases and is defined by lack of estrogen receptor (ER) and progesterone receptor (PR) expression as well as human EGFR2 (HER2) amplification [3, 4]. TNBC is more aggressive than other types of breast cancer, usually presenting larger size, higher grade, and more lymph node metastasis at diagnosis [3, 5, 8]. Presently there are no targeted therapies for TNBC and chemotherapy is the only choice of systemic treatment available. Notably, TNBCs are highly heterogeneous, and the same NAC chemotherapy regimen yield diverse responses. It is therefore urgent to develop clinically applicable biomarkers for TNBC to predict the response to NAC.

More than half of the human genome is known to be transcribed with the most abundant species, long noncoding RNAs (lncRNAs), these being defined as RNA transcripts larger than 200nt without coding capacity [9, 10]. Indeed according to the Encyclopedia of DNA Elements (ENCODE) project, more than 16000 transcribed lncRNAs from human genome have been identified [11]. lncRNAs are principally regulatory molecules and have been shown to participate in a diverse array of biological functions. Not surprisingly, aberrant expression of lncRNAs occurs in disease and notably certain lncRNAs have been strongly implicated in the pathology of breast cancer [11-13]. lncRNA HOTAIR, for example, binds the polycomb repressive complex 2 to silence genes involved in chromatin remodeling [14, 15]. Compared with coding genes, lncRNAs are expressed in a more temporal and spatially-specific manner, indicating their potential to serve as novel biomarkers [16, 17].

In the current study, we aimed to analyse the expression of coding and non-coding RNAs in TNBC and employ their intersection to develop a response score that would predict which TNBC patients will undergo pCR in response to NAC.

Materials and Methods

Gene expression profile of TNBC patients receiving NAC

We searched the publically available Gene Expression Omnibus (GEO) database to identify datasets where TNBC patients received NAC. Only datasets from the Affymetrix human genome U133 plus 2 microarray platform were interrogated, since it is widely used and includes 54000 probe sets that cover most region of human genome. Datasets were filtered according to the following criteria: 1) expression assay was performed with HG-U133 plus2 microarray, 2) TNBC patients received NAC, 3) data included about ER, PR, and HER status and response to NAC, 4) ≥ 5 patients with pCR or not complete response (nCR) to NAC. Accordingly only the GSE32646 dataset [18] met these criteria. It consists of 115 breast cancer patients, of which 26 are TNBC (10 pCR, 16 nCR). Only the 26 TNBC patients were included for analysis.

Microarray data analysis and lncRNA profile

Differentially expressed lncRNAs and coding genes were calculated with the online GEO2R tool (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>). To analyse lncRNA expression we employed a pipeline to annotate HG-U133 Plus 2.0 probe set as reported previously [19]. Briefly, the NetAffx Annotation Files (HG-U133 Plus 2.0 Annotations, CSV format) were downloaded and the probe IDs were mapped to annotation files, then probe

ID-centric data was matched with annotations of database accession IDs, including RefSeq and Ensembl. Noncoding transcripts were further filtered out according to transcript types [19].

Bioinformatic analyses

Genomic location data of differentially expressed lncRNAs were submitted online to the GREAT website (<http://bejerano.stanford.edu/great/public/html/>). Enrichment of GREAT results were plotted by Cytoscape software [20]. Functional annotation (Gene Ontology (GO)) and pathway analyses of coding genes were performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) website (<https://david.ncifcrf.gov/home.jsp>). Hierarchical cluster and heat map of differentially expressed lncRNAs were conducted using Cluster 3.0 with one minus correlation and average linkage methods.

Statistical analyses

Continuous variables were compared using Student's *t* test. Receiver operating characteristic (ROC) curves were constructed to assess sensitivity, specificity, and respective area under the curve (AUC) with 95% confidential interval (CI). Statistical significance was taken as a two-tailed *P* value <0.05 unless specifically indicated. The statistical analyses were performed using SPSS (version 18.0; SPSS Institute Inc., Chicago, IL, USA).

Results

Patient characteristics

Breast cancer patients in the GSE32646 dataset received NAC regimens consisting of paclitaxel, 5-fluorouracil, epirubicin and cyclophosphamide (paclitaxel followed by 5-fluorouracil, epirubicin and cyclophosphamide [P-FEC]). According to the ER, PR, and HER2 status criteria, 26 of the 115 patients in the dataset were identified as the TNBC subtype. Patients had a median age of 54.4, staging was from IIA to IIIB and 22 patients had lymph node involvement. Ten patients had pCR, and 16 had nCR.

Differentially expressed lncRNAs between pCR and nCR patients

We set $p < 0.01$ as the threshold to filter for differentially expressed lncRNAs and coding genes. As shown, 172 coding genes and 84 lncRNAs were differentially expressed between pCR and nCR TNBC patients (Supplementary Table 1). For all supplemental material see www.karger.com/doi/10.1159/000492698. 85 coding genes were upregulated in pCR and 87 coding genes were downregulated; 39 lncRNAs were upregulated and 45 lncRNAs were downregulated in pCR compared with nCR (Fig. 1).

We then analyzed the potential functions of differentially expressed genes with functional annotation tools (GO and KEGG pathway analysis, Fig. 2). As shown, the biological processes of chromatin remodeling and regulation of axonogenesis were most significantly enriched. For the molecular function annotation results, genes with the function "drug binding" were enriched.

To predict the biological function of the differentially expressed lncRNAs, the genomic regions enrichment

Fig. 1. Heatmap of differentially expressed coding genes (A) and lncRNAs (B) between pCR and nCR TNBC patients. Red: upregulated; green: downregulated.

Fig. 2. Functional annotation of differentially expressed coding genes. A: biological process; B: cellular component; C: molecular function; D: KEGG pathway.

Fig. 3. GREAT analysis of differentially expressed lncRNAs. Many binding motifs of transcription factors are enriched nearby the genomic region of differentially expressed lncRNAs.

of annotations tool (GREAT)[21] was performed. Most differential lncRNAs were located near transcription start sites, which is consistent with previous reports that lncRNAs are actively transcribed from human genome. Additionally, the GREAT output suggests there are various binding sites of transcription factors within genomic region of lncRNAs (Fig. 3). The identified transcription factors included those known to have critical roles in breast cancer, for example FOXA1 [22], indicating these lncRNAs may also play important roles.

Development of a response score to predict response to NAC

We first filtered potential lncRNAs and mRNAs that could predict response to NAC. Differentially expressed lncRNAs or coding genes with p value <0.01 were first selected, and fold change >2 or <-2 was set to identify differentially expressed lncRNAs, and fold change >4 or <-4 was set to identify differentially expressed coding genes. For lncRNAs, we further selected those with HGNC gene symbol and transcript type of “antisense”, “lncRNA”, “processed pseudogene”, “unprocessed pseudogene”, and “transcribed unprocessed pseudogene”. This process identified 23 lncRNAs and 15 coding genes for subsequent ROC analysis (Table 1 and Table 2). ROC was performed for each lncRNA and coding gene to assess their predictive value for NAC. As shown in Table 3 and Table 4, only lncRNA BPESC1, coding genes WDR72 and GADD45A showed excellent predictive efficacy.

With these 3 genes, we developed a “Response Score” for NAC in TNBC patients, response score (RS) = $2.595 \times \text{BPESC1} - 1.09 \times \text{WDR72} - 1.428 \times \text{GADD45A} - 0.731$. As shown, the response score could significantly distinguish pCR and nCR patients with AUC of 0.931 ($p < 0.01$) (Fig. 4). Patients with high RS were more likely to have pCR. According to ROC analysis, an optimal cut-off of 0.541 was determined, and the predictive sensitivity and specificity of response score was 0.8 and 0.9, respectively. We then evaluated our response score against the entire GSE32646 dataset including non-TNBC cases. As shown, response scores between pCR and nCR were statistically different among TNBC patients (Fig. 5), whereas response scores were not statistically different between pCR and nCR in patients with tumors positive for ER, PR, or HER2 overexpression.

Table 1. 23 lncRNAs identified for ROC analysis. FC: fold change, lincRNA: large intergenic RNA

HGNC symbol	Transcript stable ID	PValue	logFC	Transcript type	Transcript length
TSPEAR-AS2	ENST00000330490	0.005334	2.542854	antisense	823
FAR1P1	ENST00000427163	0.001067	1.896832	processed_pseudogene	934
TPT1P8	ENST00000510120	0.002824	1.718356	unprocessed_pseudogene	555
ANP32C	ENST00000512835	0.005074	1.08722	processed_pseudogene	705
CLSTN2-AS1	ENST00000513309	0.005882	0.971401	antisense	2693
LINC01857	ENST00000429730	0.001097	0.813998	lincRNA	492
LINC01588	ENST00000623502	0.004206	0.749833	lincRNA	975
BPESC1	ENST00000418282	0.002015	0.724564	lincRNA	3522
LINC00452	ENST00000609661	0.007386	0.629585	lincRNA	2896
LINC02387	ENST00000541749	0.008513	0.570136	lincRNA	868
L3MBTL4-AS1	ENST00000578427	0.00256	-0.64378	lincRNA	2044
SPATA41	ENST00000558307	0.002081	-0.80954	lincRNA	1252
MST1P2	ENST00000457982	0.002379	-0.90345	unprocessed_pseudogene	2291
RBMY2BP	ENST00000451071	0.009828	-0.98378	transcribed_unprocessed_pseudogene	1156
LINC01592	ENST00000518540	0.008176	-1.01895	lincRNA	2367
NFIA-AS2	ENST00000438559	0.008628	-1.23862	antisense	1275
CSMD2-AS1	ENST00000425631	0.009892	-1.29474	antisense	1367
UBBP2	ENST00000376781	0.007114	-1.31044	processed_pseudogene	231
LINC00593	ENST00000558385	0.008152	-1.31972	lincRNA	763
GAS5	ENST00000449289	0.008703	-1.36826	lincRNA	542
SOX1-OT	ENST00000421423	0.006632	-1.62168	lincRNA	545
FAM53B-AS1	ENST00000448422	0.007983	-1.71488	antisense	1491
LINC01847	ENST00000522627	0.000654	-2.54338	antisense	5607

Table 2. 15 coding genes identified for ROC analysis. FC: fold change

HGNC symbol	logFC	Transcript stable ID	Chromosome	Transcript length
PCDHB1	2.926422	ENST00000306549	chr5	8210
RGS11	2.650471	ENST00000397770	chr16	2390
GADD45A	2.394995	ENST00000370986	chr1	1496
ZNF614	2.372718	ENST00000356322	chr19	1293
NEURL4	2.224102	ENST00000315614	chr17	5182
HNRNPA0	2.18639	ENST00000314940	chr5	8726
C4orf3	2.021667	ENST00000399075	chr4	2960
PCOTH	-2.02016	ENST00000382133	chr13	805
CABP4	-2.14612	ENST00000438189	chr11	1426
S100A12	-2.18748	ENST00000368737	chr1	518
MED14	-2.20079	ENST00000324817	chrX	7984
KIAA1210	-2.32596	ENST00000402510	chrX	7824
CCL15	-2.42104	ENST00000631730	Chr7	718
COL20A1	-2.43183	ENST00000422202	chr20	8097
WDR72	-2.4617	ENST00000396328	chr15	7507

Table 3. AUC of 23 lncRNAs. AUC: area under curve, *significant association

lncRNA	Area	Std. Error	P value	95% CI
NFIA-AS2	0.494	0.118	0.958	0.263-0.724
SOX1-OT	0.513	0.114	0.916	0.289-0.736
LINC01847	0.631	0.115	0.268	0.405-0.858
FAM53B-AS1	0.513	0.133	0.916	0.252-0.773
GAS5	0.631	0.113	0.268	0.41-0.853
FAR1P1	0.606	0.115	0.37	0.381-0.831
CSMD2-AS1	0.587	0.114	0.461	0.365-0.81
LINC01592	0.588	0.113	0.461	0.365-0.81
LINC00593	0.481	0.116	0.874	0.254-0.709
UBBP2	0.55	0.119	0.673	0.317-0.783
TSPEAR-AS2	0.594	0.117	0.429	0.364-0.823
ANP32C	0.638	0.115	0.246	0.412-0.863
BPESC1	0.825	0.093	0.006*	0.635-1
LINC01588	0.562	0.116	0.598	0.336-0.789
LINC00452	0.531	0.118	0.792	0.3-0.763
RBMV2BP	0.575	0.115	0.527	0.349-0.801
LINC02387	0.562	0.123	0.598	0.321-0.804
L3MBTL4AS1	0.631	0.116	0.268	0.404-0.858
SPATA41	0.619	0.123	0.317	0.378-0.859
LINC01857	0.475	0.115	0.833	0.25-0.7
MST1P2	0.556	0.115	0.635	0.331-0.781
CLSTN2AS1	0.575	0.114	0.527	0.352-0.798
TPT1P8	0.687	0.109	0.114	0.473-0.902

Table 4. AUC of 15 coding genes. AUC: area under curve, *significant association

Gene	AUC	Std. Error	P value	95% CI
GADD45A	0.794	0.094	0.013*	0.609-0.978
ZNF614	0.531	0.117	0.792	0.303-0.76
KIAA1210	0.481	0.117	0.874	0.252-0.711
HNRNPA0	0.481	0.118	0.874	0.249-0.713
CABP4	0.756	0.097	0.031	0.566-0.947
NEURL4	0.612	0.114	0.343	0.39-0.835
PCOTH	0.631	0.11	0.268	0.416-0.847
MED14	0.563	0.127	0.598	0.313-0.812
RGS11	0.525	0.122	0.833	0.285-0.765
C4orf3	0.488	0.128	0.916	0.238-0.737
S100A12	0.494	0.12	0.958	0.259-0.729
PCDHB1	0.5	0.116	1	0.272-0.728
CCL15	0.506	0.117	0.958	0.278-0.735
WDR72	0.756	0.096	0.031*	0.569-0.944
COL20A1	0.594	0.115	0.429	0.368-0.819

Fig. 4. Predictive efficacy of response score. Distribution of response score in 26 TNBC patients (A). For response, red represents pCR, green represents nCR. (B) ROC analyses of response score, WDR72, GADD45A, and BPESC1, respectively.

Fig. 5. Response score distribution among patients with different ER, PR, and HER2 status. N.S: not statistically significant; *P<0.05; **P<0.01.

Discussion

TNBC is an aggressive type of breast cancer with poor prognosis. It is reported about 30% of TNBC patients will eventually relapse and fail to response to conventional chemotherapies [3]. Numerous efforts have been made to develop tools for prediction of response to NAC in order to optimize chemotherapy for individuals. Some conventional clinical parameters may predict response to NAC, and a nomogram has been built [23]. Moreover, similar methods to our study have been applied before to predict pCR in NAC. Nevertheless, the predictive value of lncRNAs in TNBC patients treated with NAC has not been fully explored and our approach may provide some advantages.

Here, we developed “Response Score”, consisting of just one lncRNA and two coding genes for TNBC patients with NAC. The response score showed highly predictive efficacy in GSE32646 dataset (AUC=0.931). Wang et al. [24] previously developed a 36-lncRNA signature to predict NAC response in breast cancer, while the specific predictive efficacy was not determined in TNBC subgroup. Jiang et al. [25] also developed a signature consisting of 3 coding genes (FCGR1A, RSAD2, CHRDL1) and 2 lncRNAs (HIF1A-AS2, AK124454) to predict NAC response in TNBC patients. Compared with Jiang, our response score consists of one lncRNA and two coding genes which as a small gene signature may be more clinically feasible to measure with conventional instrumentation and approaches. However, the main shortcoming of our study is that our response score was not independently validated in other datasets and further validation is therefore needed.

Our response score consists of 2 coding genes, GADD45A and WDR72. GADD45A is a member of a group of genes whose transcript levels are increased following stressful growth arrest conditions and treatment with DNA-damaging agents. Many chemotherapeutic agents act through inducing DNA damage including those used here as NAC (5-fluorouracil, epirubicin and cyclophosphamide). GADD45A functions in DNA repair and it has also been confirmed it plays a role in promoting DNA methylation [26, 27]. On this basis, GADD45A is a logical candidate to show predictive efficacy to NAC (Table 4). In addition to GADD45A, GO analysis showed genes involved in chromatin remodeling were enriched among differentially expressed coding genes, suggesting epigenetic modification may play important roles in chemotherapy resistance. On the other hand, the exact function of WDR72 is still unknown. The WDR72 protein can act as a protein scaffold for protein-protein interactions and mutation of WDR72 is associated with amelogenesis imperfect. The role of WDR72 in cancers has been poorly investigated. It is reported that WDR72 is involved in a gene signature of 19 genes, which could be used for diagnosis of esophageal cancer [28]. This suggests WDR72 might be a sensitive biomarker.

To identify lncRNAs of predictive value, our filtering approach yielded 23 lncRNAs for ROC analysis (Table 1). Among the 23 lncRNAs shortlisted only a few have been investigated in detail such as GAS5 [29] while the functions of most others like lncRNA BPESC1 are presently unknown. We included lncRNA BPESC1 in the response score since it showed excellent predictive efficacy. According to RefSeq [30], BPESC1 is located on chromosome 3, and is transcribed as 3518nt from 3 exons and contains an open reading frame of 351 bp. The functional role of BPESC1 has not been investigated in breast cancer and further exploration is needed.

Conclusion

We profiled coding gene and lncRNA expressions in TNBC patients receiving NAC and developed a response score consisting of lncRNA BPESC1 and coding genes WDR72 and GADD45A. The response score efficiently predicted TNBC patients with pCR to NAC.

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Disclosure Statement

The authors declare no conflict of interests.

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