NUSAP1 and KIAA0101 downregulation by neo-adjuvant therapy is associated with better outcome and survival in breast cancer.

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Research

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Abstract

Background: Studies of molecular changes occurring before and after neo-adjuvant chemotherapy (NCT) for breast cancer may unveil genetic biomarkers to predict therapy response. This study aimed at identifying genomic changes in breast primary tumors of patients under NCT. Gene expression changes were correlated with pathological response and survival.

Methods: Gene expression profiles in tissue samples from pre and post NCT were obtained by a non-supervised classification analysis. Thirty-nine patients were classified according to their response to the chemotherapy as pathologic complete responders or non-responders (pCR and no-pCR, respectively). Overall survival was assessed by comparing gene expression values before NCT using the Log-rank (Mantel-Cox) test.

Results: A signature constituted by 43 genes was obtained to stratify pCR and no-pCR patients after NCT (FC = +3, FDR p-value < 0.0298). These genes were involved in regulation of the mitotic nuclear division and the anaphase-promoting complex-dependent catabolic process. Remarkably, over-expression of NUSAP1 and KIAA0101 were associated to poor overall survival.

Conclusions: A new expression signature evaluating response for the neo-adjuvant chemotherapy stratified pathological response. The expression levels of NUSAP1 and KIAA0101 before and after the neo-adjuvant therapy may be useful to predict overall survival.

Background

Clinical factors like age [1], clinical stage [2], and histopathological and molecular subtypes [3] are predictors for treatment response and prognosis in breast cancer (BC). Genomic signatures can provide additional information on the biological behavior of a tumor and serve to calculate the risk of relapse and the survival, helping to define the best treatment options [4-6]. Genomic signatures allow identifying gene expression patterns related to chemoresistance, immune system response, and invasion [7-10]. Commercial genomic signature screening is amenable for identifying low-risk biology tumors associated with favorable prognosis at 3 and 5 years and help to solve therapeutic decisions for aggressive tumors with poor response to conventional therapies.

Gene expression comparisons of biopsy specimens taken after and before neo-adjuvant chemotherapy treatment (NCT), maybe useful to define the tumor molecular adaptations to a specific therapeutic agent or regime [7-10]. During the course of the neo-adjuvant treatment, the achievement of a pathological complete response (pCR) correlates with patient survival [11]. Specific treatment regimens have been shown to improve survival when a pCR is not achieved. A pCR also identifies individuals that could benefit from different treatments in case of non-response [12]. Comparisons of the changing patterns of gene signatures in response to chemotherapy, may enable predictions of clinical response and prognosis, and sometimes, to recognize new response biomarkers of specific pathways related to treatment resistance and recurrence.
The objective of this work was to observe and analyze the genomic adaptations in the primary tumor of patients with BC to the effects of NCT, and to correlate these changes with pathological response and prognosis.

Materials And Methods

Patient population. BC patients were recruited, consented, and enrolled in the study in the Breast Cancer Center of the San Jose Hospital in Monterrey, Mexico. The Institutional Review Board from the School of Medicine of Tecnologico de Monterrey authorized the research protocol with the number: P000088-Altru-Pro-Ci-CR002. Tissue samples from 54 patients with clinical and/or radiological diagnosis of BC (tumor size > 2 cm and palpable lymph nodes) were collected from July 2011 to October 2014.

Neo-adjuvant chemotherapy regimens. Regimens were established according to the clinical stage and the immunohistochemistry of the breast tumors by medical oncologists. They consisted of 4 cycles every 3 weeks of either cyclophosphamide intravenous (IV) (500-1500 mg/m$^2$) and doxorubicin IV (≥ 40 mg/m$^2$) or cyclophosphamide IV (500-1500 mg/m$^2$) and epirubicin IV (≥ 60 mg/m$^2$). After receiving any of these schemes, the patients received 12 weekly cycles of paclitaxel IV infusions (80 mg/m$^2$) administered in 1 hr. In patients who presented drug toxicity, cycles of carboplatin replaced the drug responsible for the toxicity. After NCT, surgical resection of the breast was performed in all patients.

Tumor sample collection. Two tissue samples were collected from each patient: a biopsy sample (BS) before NCT paired with tissue after completing the NCT cycles (surgery sample or SS). Thick needle puncture biopsies were obtained using a Bard Magnun 12 Fr gauge Needle (Bard®). Tumor location was marked at diagnosis using a carbon tracking technique [13]. Six to eight tissue cylinders were obtained from each patient. Four samples were used for histopathological analysis and three samples were preserved in RNA-later solution for analysis. The SS were obtained from surgeries for locoregional control (modified radical mastectomy in most of the cases). Tissues were sent to pathology for histopathological analysis. A 2 x 1 cm piece, marked by the carbon track used during the diagnostic biopsy procedure, was preserved in RNA-later solution for the gene expression analysis.

Treatment Response. Two pathologists evaluated surgical specimens and assessed tumor response to NCT using the Miller-Payne grading system. For purposes of this study, a grade 5 score in the grading system was considered as pathological complete response (pCR), and the remaining scores (including partial pathological response) were classified as non-pathological complete responses (no-pCR) [14].

RNA isolation and Microarrays analysis (expression profiles). RNA extraction from BS and SS were done using RNeasy Fibrous Tissue Mini Kit (Qiagen, Germantown, MA) following manufacturer’s instructions. RNA quality was assessed by capillary electrophoresis using the Experion™ Automated Electrophoresis Station (Bio-Rad, CA, USA). Sample processing, microarray hybridization and gene expression analysis from the selected RNAs was conducted using the GeneChip 3'IVT Express Kit and GeneChip Human
Genome U133 Plus 2 (Affymetrix, Santa Clara, CA), according to manufacturer's instructions and previously described [15].

**Microarray data processing.** Normalization was performed using RMA (Robust Microarray Analysis) normalization. Samples from five patients were removed because they showed clearly altered profiles compared to the others (abnormal microarray quality controls), leaving 39 patients for this study. Probes with mean expression lower than 3 (in logarithmic scale resulted from RMA) were also removed.

Differential gene expression signature was performed using t-test with multiple comparison corrections using the False Discovery Rate method (FDR) [16]. We considered as positive the probes with a significant p-value (FDR: \( p < 0.05 \)). These analyses were performed using the free Transcriptome Analysis Console (TAC) 4.0.1 software from Applied Biosystems (Thermo Fisher Scientific, Waltham, MA).

**Gene network.** An interaction analysis of the selected genes was carried out using the online tool STRING: functional protein association networks version 11.0 [17]. The combined score was computed by combining the probabilities from the different evidence channels and corrected for the probability of randomly observing an interaction [18].

**Real-time qPCR validation.** In order to validate microarray data, four genes were selected based on the consistency of their expression in the different analyses: two over-expressed (\textit{MME} and \textit{DST}) and two under-expressed (\textit{NUSAP1} and \textit{KIAA0101}). \textit{GRAMD1A} was used as an endogenous gene control due to a low variation between the samples [15]. Expression analyses of these genes, as well as one endogenous control (\textit{GRAMD1A}), were assessed using predesigned hydrolysis probes (\textit{MME}: Hs00153510\_m\_1, \textit{DST}: Hs00156137\_m\_1, \textit{NUSAP1}: Hs01006195\_m\_1, \textit{KIAA0101}: Hs00207134\_m\_1, \textit{GRAMD1A}: Hs.PT.5840681431) (Thermo-Fisher Scientific). Total RNA aliquots used for microarray assays were analyzed through qPCR using Quant Studio 3 (Applied Biosystem). \( Ct \) means for each gene were used for \( dCt \) (Problem minus endogenous), and \( 2-dCt \) analysis was done using calculated \( dCt \) for all genes. In order to compare gene expression of pCR and no-pCR groups, the relative expression \( 2-dCt \) was evaluated from qPCR data of all genes after normalization with \textit{GRAMD1A}. Unpaired \( T \) test with Welch´s correction was used (\( p \)-value < 0.05) to establish differences.

**Overall survival.** Comparison of BS gene expression values with overall survival (OS) was evaluated in the 39 patients. In order to evaluate differences in OS, Log-rank (Mantel-Cox) test was used for comparison of Kaplan-Meier survival curves using GraphPad Prism version 6.01 for Windows, GraphPad Software (La Jolla, CA, [www.graphpad.com](http://www.graphpad.com)). A \( p \)-value \( \leq 0.05 \) was considered significant in all statistical analyses.

For external validation, Kaplan-Meier Plotter (http://kmplot.com/) online database [19] was used to analyze the OS correlated to high versus low gene mRNA expression levels. The Kaplan-Meier Plotter split the BC patient (\( n = 1,402 \)) samples into two groups according to their median mRNA levels. The Affymetrix probe ID used for the Kaplan Meier were \textit{KIAA0101/PCLAF} 202503\_s\_at and \textit{NUSAP1} 219978\_s\_at.
Results

Patients. Fifty-four patients were enrolled in the study, but only 44-paired samples (BS and SS) satisfied the RNA quality and quantity standards needed for microarray analysis. After this, five sets of samples were eliminated because failed to achieve quality standards; leaving 39 patient sample sets for the final analysis. Demographics and clinical characteristics of the patients are described in Table 1. Only 8 (20.5%) out of the 39 patients reached pCR, according to the Miller-Payne grading system.

Table 1. Patient characteristics.

|                              | All patients (n = 39) | pCR (n = 8) (20.5%) | no-pCR (n = 31) (71.5%) |
|------------------------------|-----------------------|---------------------|------------------------|
| Age at diagnosis (years)     | 48                    | 47                  | 48                     |
| BMI (body mass index, kg/m²) | 28.21                 | 28.4                | 28.17                  |
| <25                          | 8                     | 1                   | 7                      |
| ≥25                          | 28                    | 6                   | 22                     |
| Menopause status             |                       |                     |                        |
| Pre                          | 21                    | 5                   | 16                     |
| Post                         | 18                    | 3                   | 15                     |
| Family history               |                       |                     |                        |
| Yes                          | 19                    | 3                   | 16                     |
| No                           | 20                    | 5                   | 15                     |
| Diabetes mellitus            |                       |                     |                        |
| Yes                          | 2                     | 0                   | 2                      |
| No                           | 37                    | 8                   | 29                     |
| Number of children           |                       |                     |                        |
| Nulliparous                  | 4                     | 0                   | 4                      |
| 1 or 2                       | 12                    | 2                   | 10                     |
| >3                           | 23                    | 6                   | 17                     |
| Lactation                    |                       |                     |                        |
| Yes                          | 16                    | 3                   | 13                     |
| No                           | 11                    | 2                   | 9                      |
| Smoking                      |                       |                     |                        |
| Yes                          | 5                     | 2                   | 3                      |
| No                           | 34                    | 6                   | 28                     |
| Clinical stage               |                       |                     |                        |
| I                            | 1                     | 1                   | 0                      |
| II                           | 19                    | 2                   | 17                     |
| III                          | 19                    | 5                   | 14                     |
| Molecular subtype            |                       |                     |                        |
| Luminal A                    | 10                    | 1                   | 9                      |
| Luminal B                    | 7                     | 1                   | 6                      |
| Her2                         | 6                     | 4                   | 2                      |
| Triple negative              | 16                    | 2                   | 14                     |
**Gene expression profile analysis.** Different comparisons between pCR and no-pCR sets were made to evaluate the molecular gene expression modifications induced by NTC (Figure 1). The first comparison assessed changes induced by NCT in samples of patients achieving pCR (BS=8 vs SS=8). A profile of 21 probes representing 14 genes was found (FC = +3, FDR p-value < 0.05) (Figure 1A). Three genes were overexpressed (TOP2A, RRM2, and CDKN3) and eleven were downregulated (EGR2, ADAMTS5, JUN, APOLD1, DUSP1, CYR61, ATF3, EGR1, PTGS2, RGS1, and FOSB) (Supplementary Figure 1). Likewise, the study of the no-pCR subset of patients (BS, N=31 vs SS, N=31) identified four overexpressed genes in SS biopsies (NAMPT, DUSP1, RGS1, and FOS) (FC = +3, FDR p-value < 0.05) (Figure 1B and Supplementary Figure 2).

Lastly, comparisons between pCR and no-pCR were made in the BS (Supplementary figure 3) and in the SS groups (Figure 1C). A significant non-supervised sample clustering was only achieved in the SS subset (Figure 1D). The comparison of pCR (n = 8) vs no-pCR (n = 31) revealed a profile of 55 probes corresponding to 43 genes (FC = +3, FDR p-value < 0.0298) (Figure 1D). Thirty genes were over-expressed in patients with pCR (ABCA9, ABCB5, ADAMTS5, ANKRD29, ARHGAP20, ASPA, CCDC178, CCDC8, CD300LG, CLDN5, CNN1, COL6A6, DST, FAM196B, FHL1, FIGF; PIR-FIGF, GAS1RR, GPRASP1, HAS3, ITIH5, LRFN5, LRRN4C, MME, OXTR, PGM5, PRRG3, SCN3A, SCN4B, SDPR, and TUBB2B), while thirteen were down-regulated (ATAD2, AURKA, CCNB1, HIST1H2BD, HN1, KIAA0101, NUSAP1, PMAIP1, RRM2, SORD, TPDS2, UBE2C, and UBE2T) (Figure 2).

**Gene Network.** The online tool “STRING: functional protein association networks version 11.0” [17] was used to investigate interactions between the genes that were identified as differentially expressed in each comparison. The value of the interaction network was significant (PPI enrichment p-value: 2.26E-10), meaning that these proteins have more interactions among themselves than what would be expected from a random set of proteins of similar size drawn from the genome. Figure 1 shows the gene network for each gene expression profile. According to the protein interaction analysis, AURKA, CCDC8, CCNB1, NUSAP1, and UBE2C are involved in the regulation of nuclear division during mitosis, while AURKA, CCNB1, and UBE2C genes are part of the anaphase promoter complex dependent catabolic process.

**qPCR Validation,** DST, MME, NUSAP1 and KIAA0101/PCLAF were used to validate the microarrays by qPCR. Only 31 samples (pCR=5, no-pCR=26) out of 39 had enough quality and quantity of total RNA to perform qPCR validation analysis. Supplementary Figure 4 shows the box plot of NUSAP1 and KIAA0101 expression by qPCR (4A and 4B) and by microarray (4C and 4D). A similar analysis was made for DST and MME (supplementary Figure 4E-F). This qPCR analysis corroborated the expression patterns of all the differentially expressed genes revealed by the microarray.

**NUSAP1 and KIAA0101 gene expression.** In the subset of patients achieving pCR, NUSAP1 and KIAA0101 gene expressions were higher in the BS compared to the SS (Two way-ANOVA, F = 22.12, p-value = 0.0053) (Figure 3A and 3C); while there was no significant difference in the expression values in the no-pCR groups (Two way-ANOVA, F = 1.246, p-value = 0.2739) (Figure 3B and 3D). NUSAP1 gene expression
after NTC was overexpressed in luminal B subtype tumors compared to the other BC subtypes (F test = 4.88, p value = 0.0063) (Supplementary Figure 5).

**Overall survival.** The primary outcome variable was death due to BC. Patients were followed up for 46.5 months in average (SD=20.34, range 5.1-79.2 months). Higher levels of *NUSAP1* gene expression in the BS (56 to 89%), were associated with a decreased OS (Log-rank Mantel-Cox test, Chi square = 4.517, p-value = 0.0336) (Figure 4A). Similarly, *KIAA0101* gene overexpression negatively impacted OS, with a reduction from 84 to 60% (Log-rank Mantel-Cox test, Chi square = 2.827, p-value = 0.0927(Figure 4B). Consistent results were observed when analyzing public data from 1,402 patients from the Kaplan-Meier Plotter website (http://kmplot.com). Low levels of *NUSAP1* and *KIAA0101* were associated with greater OS (Log-rank HR = 1.82 CI = 1.46-2.26 p-value = 6.2e-08 and Log-rank HR = 1.47 CI=1.19-1.82 p-value=0.00039) (Figures 4C and 4D, respectively).

**Discussion**

High-throughput genomic technologies like microarray and mass sequencing platforms may influence the understanding of the changes in tumor biology of BC lesions after NCT. Usually, clinicians rely only in the histopathological assessment performed in biopsies and surgical materials to predict patient's prognosis. A better understanding of the tumor response to chemotherapy is important to design better treatment regimens for aggressive tumors.

In this study, tumor tissue samples of patients with BC were collected before (BS) and after neo-adjuvant therapy (SS), and then, gene expression assays were evaluated in a microarray platform. Comparison of gene expression profiles in patients who did or did not respond to treatment (pCR vs. no-pCR) provided a gene signature constituted by 43 differentially expressed genes in the SS sample. Validations with pCR corroborated the observations, confirming that 30 genes were over-expressed and 13 were under-expressed in these patients (Supplementary Table 1), some of these genes are involved in the regulation of the nuclear division during mitosis or participate in the anaphase promoter complex dependent catabolic process.

Deregulated gene expression of some of these genes has been implicated in BC. For example *CCNB1, KIAA0101, NUSAP1, RRM2, UBE2C,* and *UBE2T* alterations are part of a gene signature identified in the tumor genesis process in young women from the Middle East [20]. *NUSAP1* and *KIAA0101* were over-expressed in DCIS (ductal carcinoma in situ) and IDC (invasive ductal carcinoma) when compared to normal age-matched controls. *CCNB1, RRM2, and UBE2C* genes are reported in the PAM50 signature as elements for the molecular classification of BC lesions [21]. However, to our knowledge, the 43 gene signature described here for predicting BC response after NTC has not been reported.

*KIAA0101* and *NUSAP1* genes were found to be down regulated by the NTC in patients with pCR. Furthermore, higher expression levels of these same genes in the tumor biopsy before treatment (BS) were related to worse survival, indicating that they are potential predictors of survival in diagnostic biopsies. The protein codified by *KIAA0101* (aka *PCLAF* or PCNA Clamp Associated Factor) is a PCNA
binding protein which acts as a regulator of the number of centrosomes and it’s involved in repair mechanisms during DNA replication [22]. Over-expressed \textit{KIAA0101} has been associated with a decreased survival in BC patients [22], but not with the pathological response to NCT. \textit{NUSAP1} gene expression levels showed a remarkable inverse correlation with survival (Fig 4A and 4C). This gene encodes the nucleolar-spindle associated protein that binds to chromatin and microtubules, and is critical for the cytokinesis spindle assembly during mitosis [23]. \textit{NUSAP1} overexpression has been described in bladder, cervix, colon, glioblastoma, liver, lung, oral squamous cell carcinoma, prostrate, kidney, and breast [24-39] cancers. Multiple studies have correlated its over-expression with a poor prognosis [22, 28, 30, 32, 36, 37, 40, 41]. Zhang et al. [37] demonstrated that down-regulation of \textit{NUSAP1} suppressed proliferation, migration and invasion of MCF7 cells by disturbing the regulation of \textit{CDK1} and \textit{DLGAP5}, and reported increased susceptibility to epirubicin [37].

Both, \textit{NUSAP1} and \textit{KIAA0101}, are associated with DNA repair mechanisms through their relationship with \textit{BCRA1}. \textit{NUSAP1} promotes increased expression of the BRCA1 protein [42]. \textit{KIAA0101} regulates the number of centrosomes through its interaction with \textit{BRCA1} [22]. Since the biological role of \textit{NUSAP1} and \textit{KIAA0101} involve cell cycle pathways, patients with elevated levels of these genes can benefit from chemotherapeutic drugs interfering with \textit{BRCA1} DNA repairing pathways such as platinum derivatives. In addition, levels of expression of these genes after NCT in patients not reaching pCR may suggest a second-line therapy.

Galiellalactone (GL), a fungal metabolite with anti-tumor and anti-inflammatory properties, downregulates \textit{NUSAP1} in the DU145 by targeting the NF-\kappaB and STAT3 pathways, inducing arrest of the cell cycle. \textit{NUSAP1} overexpression may be a target for GL [43]. Another option to target \textit{NUSAP1} overexpression is the antitumor compound isopicrinin, isolated from the \textit{Rhazya stricta} plant, which has cytotoxic activity by inhibiting the assembly of microtubules [44].

The under-expression of \textit{NUSAP1} seems to sensitize osteosarcoma cells to treatment with taxol, since \textit{NUSAP1} interacts with the SUMO E3 ligase complex contributing to adequate chromosomal segregation [45]. In oral squamous cell carcinoma, \textit{NUSAP1} knockdown has been observed to potentiate paclitaxel-induced apoptosis [46]. In our case, the under-expression of \textit{NUSAP1} before NTC treatment was shown to be associated with better survival and pCR.

**Conclusions**

Differential expression analysis between pCR and no-pCR patients showed a profile constituted by 43 differentially expressed genes in tumor tissue samples collected after chemotherapy. Among these, over-expression of \textit{NUSAP1} and \textit{KIAA0101} was associated with poor prognosis in BC and with pathological response, opening the option for these genes to be used as prognostic markers of response to the NCT. Furthermore, drugs that impair the cell cycle and DNA repair mechanisms or microtubule assembly during mitosis are potential candidates for a second line of treatment in those patients who do not reach the pCR after NCT.
Abbreviations

BC: Breast Cancer.

NCT: Neo-adjuvant Chemotherapy Treatment.

pCR: Pathological Complete Response.

no-pCR: No Pathological Complete Response.

IV: Intravenous.

BS: Biopsy sample.

SS: Surgery sample.

RMA: Robust Microarray Analysis.

FDR: False Discovery Rate.

TAC: Transcriptome Analysis Console.

Declarations

Ethics approval and consent to participate. The research protocol and informed consent for participants for this study were authorized by the Ethics and Research Committees of the School of Medicine and Health Sciences of Tecnologico de Monterrey with the number P000088-Altru-Pro-CI-CR002.

Consent for publication. Study participants signed a consent form authorizing the use of individual person's data for scientific publications.

Availability of data and materials. The dataset generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. GI M-G participated in the conception and design of the study, recruited patients, collected clinical data, interpreted and analyzed data, participated in the manuscript drafting, and contributed with a critical revision of the article. SK S-F participated in the conception and design of the study, performed the molecular biology experiments, interpreted and analyzed data, participated in the
manuscript drafting, and contributed with a critical revision of the article. AF V-V contributed with the molecular biology experiments and interpreted and analyzed data. S C-H contributed with the design of the study, recruited patients, collected clinical data, and interpreted and analyzed data. P R-F contributed with data interpretation and analysis and in the manuscript drafting. J H-S-C participated in data interpretation and analysis and in the manuscript drafting. YX P-P participated in the molecular biology experiments and in the data interpretation and analysis. GS G-M contributed with the pathological analysis of biopsies and tumor samples and in the data analysis process. D D-G helped to recruit patients, collected clinical data, and participated in the data interpretation and analysis. J V-G contributed with the conception of the study and participated in the data interpretation and analysis. G B-S contributed with the molecular biology experiments and interpreted and analyzed data. A R-M participated in the interpretation and data analysis process, contributed to the drafting of the manuscript, and contributed with a critical revision of the article. R O-L participated in the conception and design of the study, in the molecular biology experiments, in the data analysis and interpretation in drafting the article, in performing a critical revision of the article and approved the final version to be published.

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Figures

Figure 1
Venn’s Diagrams and Gene Networks. A. Gene profile (14 genes) of pre and post treatment samples of pCR patients; Left up: Venn’s diagram of comparison, green circle BS and red circle SS of pCR patients; B. Gene profile (4 genes) of pre and post treatment samples of no-pCR patients; Left up: Venn’s diagram of comparison, yellow circle BS and blue circle SS of no-pCR patients; C. Gene profile (21 genes) of pre treatment samples between pCR and no-pCR patients; Left up: Venn’s diagram of comparison, green circle BS of pCR patients and yellow circle BS of no-pCR patients; D. Gene profile (43 genes) of post treatment samples between pCR and no-pCR patients; Left up: Venn’s diagram of comparison, red circle SS of pCR patients and blue circle SS of no-pCR patients. A, B, C, and D: Right up: Gene Network of gene profile; Network nodes represent proteins; Colored nodes: query proteins and first shell of interactors; White nodes: second shell of interactors; Empty nodes: proteins of unknown 3D structure; Filled nodes: some 3D structure is known or predicted; Edges represent protein-protein associations; Light blue and pink lines represent known Interactions from curated databases and experimentally determined; Green, red and dark blue lines represent predicted Interactions: gene neighborhood, gene fusions and gene co-occurrence, respectively; Yellow, black and purple lines represent other interactions: textmining, co-expression, and protein homology, respectively. Down: Gene Ontology analysis of the 5 most relevant biological process that involved the gene profile. BS=Biopsy sample (pre treatment); SS= Surgical sample (post treatment); pCR= pathologic complete response; no-pCR= no pathologic complete response.

Figure 2
Heat map of differential gene expression profile in SS (pCR vs no-pCR). Blue areas represent low gene expression, while red represents high gene expression. The top row separates the no-pCR (blue) and pCR (red) patients. Each column represents a different sample and each row, a single probe. Official gene or probe symbols are displayed at the right-side margin.

Figure 3

NUSAP1 and KIAA0101 gene expression. A and B: NUSAP1 gene expression in BS and SS in pCR (A) and no-pCR (B) patients, respectively. C and D: KIAA0101 gene expression in BS and SS in pCR (C) and no-pCR (D) patients, respectively. Blue lines and triangles: triple negative molecular subtype; red lines and squares: luminal A/B molecular subtype; green lines and circles: HER2 molecular subtypes. Two way-ANOVA was performed and p-value < 0.05 was considered as significant.
Figure 4

NUSAP1 and KIAA0101 survival curves. A and B: Survival curves for NUSAP1 and KIAA0101 considering patient gene expression for these genes before NCT (BS), respectively. Blue lines: under expression, red lines: overexpression. C and D: Survival curves of NUSAP1 and KIAA0101 using external databases, respectively. Black lines: under expression, red lines: overexpression.

Supplementary Files

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