Differential expression of SPC24, NDC80 kinetochore complex component in triple negative breast cancer.

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Women diagnosed with triple negative breast cancer can benefit neither from endocrine therapy nor from HER2-targeted therapies¹. We mined published microarray datasets^{2,3} to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We report here significant differential expression of the gene encoding SPC24, NDC80 kinetochore complex component, SPC24, when comparing the tumor cells of patients with triple negative breast cancer to normal mammary ductal cells². SPC24 was also differentially expressed in bulk tumor in human breast cancer³. SPC24 mRNA was present at significantly increased quantities in TNBC tumor cells relative to normal mammary ductal cells. SPC24 may be of relevance to initiation, maintenance or progression of triple negative breast cancers.

Keywords: breast cancer, SPC24, SPC24, NDC80 kinetochore complex component, systems biology of breast cancer, targeted therapeutics in breast cancer.

Patients with hormone receptor positive disease can benefit from endocrine therapy⁴: aromatase inhibition such as that provided by exemestane or letrozole, or estrogen receptor antagonism such as that provided by tamoxifen or fulvestrant. Patients whose tumors overexpress the human epidermal growth factor 2 (HER2) can be treated with HER2-targeted therapies: HER2 inhibitors, immunoglobulin⁵- or small molecule-based⁶. As triple negative breast cancer (TNBC) is defined by its lack of HER2 expression as well as by its lack of expression of the hormone receptor estrogen, patients diagnosed with triple negative breast cancer will not benefit from either of these treatment strategies.

Understanding the transcriptional composition of triple negative breast cancers in humans can facilitate development of novel therapeutics and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data^{2,3} to understand at the transcriptome level and in an unbiased fashion genes most differentially expressed in the tumor cells of patients with triple negative breast cancer.

Methods

We utilized datasets GSE38959² and GSE109169³ for this differential gene expression analysis of female breast cancer in conjunction with GEO2R. GSE38959 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=13$ samples of normal mammary gland ductal cells and $n=30$ samples of tumor cells from patients with triple negative breast cancer; analysis was performed using platform GPL4133. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast; analysis was performed using platform GPL5175. The tissues from this dataset are paired tissues (25 tumors matching 25 normal breast tissues from 25 patients). The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI-generated category of platform annotation was used. A statistical test was performed to evaluate whether SPC24 expression was significantly different between primary breast tumors and breast tissue using a two-tailed t-test.

Results

We performed discovery of genes associated with triple negative breast cancer in humans by mining two independently published microarray datasets^{2,3}.

SPC24 is differentially expressed in primary tumors and tumor cells of the breast.

Comparison of the tumor cells of patients with triple negative breast cancer to normal mammary ductal cells² revealed SPC24, NDC80 kinetochore complex component, encoded by SPC24, as among the genes whose expression was most significantly different in human TNBC as compared to the normal breast (Chart 1). When sorting each of the genes expressed in the tumor cells of patients with triple negative breast cancer based on significance of difference as compared to normal mammary ductal cells, SPC24 ranked 78 out of 45015 total transcripts, equating to 99.8% differential expression (Chart 1). Differential expression of SPC24 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 1; $p=3.30E-13$).

Analysis of a second microarray dataset³, here comparing 25 normal breast tissues to 25 tumors of the breast revealed that SPC24 was also among the genes most differentially expressed in human breast cancer, here in the tumors of women diagnosed with early-onset breast cancer (Chart 2). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, SPC24 ranked 2365 out of 19076 total transcripts, equating to 87.6% differential expression (Chart 2). Differential expression of SPC24 in female breast cancer was statistically significant (Chart 2; $p=2.24E-06$).

1 These data indicated that differential expression of SPC24 was not an artifact of a single
2 microarray dataset, was not an artifact of FACS isolation, and suggested that perturbed expression of
3 SPC24 was likely a transcriptional feature of triple negative breast cancers and early-onset human breast
4 cancers.

5 **SPC24 is expressed at significantly higher levels in triple negative tumor cells as compared to**
6 **normal cells of the breast.**

7 We obtained exact mRNA expression levels for SPC24 - from normal mammary ductal cells of the
8 breast and from tumor cells of patients with triple negative breast cancer - to understand the magnitude
9 and direction of SPC24 expression change in TNBC. SPC24 mRNA transcript was present at higher
10 levels in triple negative tumor cells of the breast as compared to normal mammary ductal cells (Figure 1).
11 Increased expression of SPC24 in primary tumor-isolated triple negative breast cancer cells was
12 statistically significant (Figure 1: $p < 0.0001$). SPC24 was expressed at 27.0 ± 47.7 arbitrary units (A.U.) in
13 normal mammary ductal cells, while it was expressed at 292.1 ± 224.1 A.U. in tumor cells of patients
14 with triple negative breast cancer. We calculated a mean fold change of 10.81 when comparing triple
15 negative breast cancer cells to normal mammary ductal cells.

16 **Discussion**

17 We performed comparative transcriptome analysis using two independently published microarray
18 datasets^{2,3} to define and catalog transcriptional differences in human triple negative breast cancer. We
19 provided evidence here that differential expression of SPC24, NDC80 kinetochore complex component,
20 encoded by the SPC24 gene, is a defining transcriptional feature of triple negative breast cancer. SPC24
21 was also differentially expressed in the bulk tumors of patients with early-onset breast cancer. SPC24,
22 NDC80 kinetochore complex component and the molecular processes to which it pertains to may be
23 relevant to the initiation or progression of triple negative breast cancer in humans.
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Rank: 78
Probe ID: 10068
p-value: 3.30E-13
t: -10.1324048
B: 19.92794
Gene: SPC24
Gene name: SPC24, NDC80 kinetochore complex component

Chart 1: SPC24 is differentially expressed in triple negative breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Rank: 2365
Probe ID: 3850660
p-value: 2.24E-06
t: -5.33
B: 4.405497
Gene: SPC24
Gene name: SPC24, NDC80 kinetochore complex component

Chart 2: SPC24 is differentially expressed in early-onset human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Figure 1: SPC24 is expressed at significantly higher levels in primary TNBC tumor cells as compared to normal cells of the breast.

The mRNA expression level of SPC24 in normal mammary ductal cells (left) and in the isolated primary tumor cells of patients with triple negative breast cancer (right) is graphically depicted with the result of a statistical test evaluating significance of difference in SPC24 expression between normal cells of the breast and TNBC tumor cells, a *p*-value, listed above.