Differential expression of LINC02716 in cancers of the breast.

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Breast cancer affects women at relatively high frequency¹. 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 LINC02716, DKFZp779M0652, when comparing primary tumors of the breast to the tissue of origin, the normal breast. DKFZp779M0652 was also differentially expressed in the brain metastases of patients with metastatic breast cancer. DKFZp779M0652 mRNA was present at significantly lower quantities in tumors of the breast as compared to normal breast tissue. DKFZp779M0652 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, DKFZp779M0652, LINC02716, systems biology of breast cancer, targeted therapeutics in breast cancer.

Invasive breast cancer is diagnosed in over a quarter of a million women in the United States each year¹ and in 2018, breast cancer was the leading cause of cancer death in women worldwide⁴. While patients with localized breast cancer are provided a 99% 5-year survival rate, patients with regional breast cancer, cancer that has spread to lymph nodes or nearby structures, are provided an 86% 5-year survival rate^{5,6}. Patients with metastasis to distant sites, like the brain, are provided a 27% 5-year survival rate^{5,6}. Understanding how primary tumors are most transcriptionally different from the tissue from which they originate, the breast, can facilitate development of novel diagnostic and therapeutics to promote early detection and enhanced treatment, 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 primary tumors of the breast as compared to normal breast tissue. LINC02716 emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE42568² and GSE43837³ for this global differential gene expression analysis of female breast cancer. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570; the majority of patients whose tumors were analyzed were age 50 or older. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary tumors of the breast and $n=19$ brain metastases from patients with breast cancer; analysis was performed using platform GPL6480. 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 DKFZp779M0652 expression was significantly different between primary breast tumors and normal breast tissue using a two-tailed t-test.

Results

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

DKFZp779M0652 is differentially expressed in primary tumors of the breast.

Studying the global gene expression profiles of 104 breast cancers from patients aged 31 to 89 revealed that the gene encoding LINC02716, DKFZp779M0652, was among the genes most differentially expressed in tumors of the breast in human breast cancer (Chart 1). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, DKFZp779M0652 ranked 80 out of 54675 total transcripts, equating to 99.9% differential expression (Chart 1). Differential expression of DKFZp779M0652 in female breast cancer was statistically significant (Chart 1; $p=3.99\text{E-}32$).

Analysis of a second microarray dataset³, here studying global gene expression patterns in the brain metastases of patients with metastatic breast cancer, again revealed significant differential expression of DKFZp779M0652 in tumor tissue of patients with human breast cancer (Chart 2). When sorting each of the genes expressed in the brain metastases of patients with breast cancer based on significance of difference as compared to normal breast tissue, DKFZp779M0652 ranked 28247 out of 61359 total transcripts, equating to 54.0% differential expression (Chart 2). Differential expression of DKFZp779M0652 in the brain metastases of patients with breast cancer was not deemed statistically significant (Chart 2; $p=0.33021462$). These data suggested that differential expression of

DKFZp779M0652 was not an artifact of a single microarray dataset, nor was it strictly associated with primary tumors of the breast, rather a reflection of the spectrum of transformation in human cancer.

DKFZp779M0652 is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for DKFZp779M0652 from the breast and from breast tumors to understand the magnitude and direction of DKFZp779M0652 expression change. DKFZp779M0652 was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of DKFZp779M0652 in primary breast tumors was statistically significant (Figure 1: $p < 0.0001$). DKFZp779M0652 was expressed at 4.75 ± 1.22 arbitrary units (AU) in normal breast tissue, while it was expressed at 2.48 ± 0.32 AU in tumors of the breast. We calculated a mean fold change of 0.52 in DKFZp779M0652 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and down-regulation of DKFZp779M0652 was among the most significant transcriptional features in primary tumors from patients with breast cancer.

Discussion

Invasive breast cancer is a medical problem with a 27% 5-year survival rate for women whose disease has spread to distant sites^{5,6}. To facilitate understanding of the basic transcriptional differences between primary tumors of the breast and the tissues from which these tumors originate, normal breast tissues, we performed comparative transcriptome analysis using two independently published microarray datasets^{2,3}, providing evidence here that differential expression of LINC02716, encoded by DKFZp779M0652, is a defining transcriptional feature of human breast cancer: in patients diagnosed after age 50, and in brain metastatic tissues from patients with human breast cancer. DKFZp779M0652 was expressed at significantly lower levels in primary tumors from patients with breast cancer as compared to normal breast tissue. LINC02716 and the molecular processes to which it pertains to may be relevant to the initiation or progression of human breast cancer.

References

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Rank: 80
Probe ID: 230115_at
p-value: 3.99E-32
t: 1.63E+01
B: 62.5180777
Gene: DKFZp779M0652
Gene name: DKFZp779M0652

Chart 1: DKFZp779M0652 is differentially expressed in the primary tumors of patients with 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: 28247
Probe ID: Hs.143715.0.A1_3p_at
p-value: 0.33021462
t: 0.9848739
B: -5.17536
Gene: DKFZp779M0652
Gene name: LINC02716

Chart 2: DKFZp779M0652 is differentially expressed in the brain metastases of patients with metastatic 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: DKFZp779M0652 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of DKFZp779M0652 in normal breast tissue (left) and in primary tumors of the breast (right) is graphically depicted with the result of a statistical test evaluating significance of difference in DKFZp779M0652 expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.