Differential expression of C-X-C motif chemokine ligand 12, CXCL12, 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 C-X-C motif chemokine ligand 12, CXCL12, when comparing primary tumors of the breast to the tissue of origin, the normal breast. CXCL12 mRNA was present at significantly lower quantities in tumors of the breast as compared to normal breast tissue. Analysis of human survival data revealed that expression of CXCL12 in primary tumors of the breast was correlated with overall survival in patients with luminal A or HER2+ cancers, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by molecular subtype. CXCL12 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, CXCL12, C-X-C motif chemokine ligand 12, 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. C-X-C motif chemokine ligand 12 emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE109169² and GSE42568³ for this differential gene expression analysis of female breast cancer. 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 first dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). 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. 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 CXCL12 expression was significantly different between primary breast tumors and breast tissue using a two-tailed t -test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of CXCL12 mRNA expression levels with overall survival (OS) in $n=404$ patients with basal subtype cancer, $n=794$ patients with luminal A subtype cancer, $n=515$ patients with luminal B subtype cancer, and $n=166$ patients with luminal A cancer.

Results

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

CXCL12 is differentially expressed in primary tumors of the breast.

Comparison of 25 normal breast tissues to 25 tumors of the breast² from patients with early-onset breast cancer revealed that the gene encoding the C-X-C motif chemokine ligand 12, CXCL12, 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, CXCL12 ranked 56 out of 19076 total transcripts, equating to 99.7% differential expression (Chart 1). Differential expression of CXCL12 in female breast cancer was statistically significant (Chart 1; $p=4.79\text{E-}16$).

Analysis of a second microarray dataset³, here in 104 tumors from patients aged 31 to 89 at diagnosis, the majority of which were above age 50, again revealed significant differential expression of CXCL12 in human breast cancer (Chart 2). When sorting each of the genes expressed in the tumors of

patients with breast cancer based on significance of difference as compared to normal breast tissue, CXCL12 ranked 2619 out of 54675 total transcripts, equating to 95.2% differential expression (Chart 2). Differential expression of CXCL12 in the tumors of patients with breast cancer was statistically significant (Chart 2; $p=1.41E-10$). These data suggested that differential expression of CXCL12 was not an artifact of a single microarray dataset, nor was it strictly associated with early-onset breast cancer, rather a general feature of cancers of the breast.

CXCL12 is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for CXCL12 from the breast and from breast tumors to understand the magnitude and direction of CXCL12 expression change. CXCL12 was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of CXCL12 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). CXCL12 was expressed at 9.98 ± 0.37 arbitrary units (AU) in normal breast tissue, while it was expressed at 8.36 ± 0.60 AU in tumors of the breast. We calculated a mean fold change of 0.84 in CXCL12 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

CXCL12 expression correlates with survival outcomes in luminal A subtype and HER2+ human breast cancers.

We performed Kaplan-Meier survival analysis in an attempt to correlate CXCL12 mRNA expression levels with survival outcomes in patients with breast cancer, evaluating correlations between molecular subtype and overall survival. We observed a significant correlation between CXCL12 expression and overall survival (OS) in patients with luminal A subtype breast cancer that approached statistical significance (Figure 2; log rank p -value: 0.054 for overall survival, hazard ratio: 0.73 (0.53-1.01) (Fig. 2)). CXCL12 mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. Median OS was 108 months for luminal A patients with low tumor expression of CXCL12 while median OS was 169.2 months for luminal A patients with high tumor expression of CXCL12 (Chart 3). We also observed a statistically significant correlation between CXCL12 expression and overall survival (OS) in patients with HER2+ breast cancer (Figure 2; log rank p -value: 0.014 for overall survival, hazard ratio: 0.49 (0.27-0.88) (Fig. 2)). CXCL12 mRNA levels were a positive prognostic indicator in HER2+ breast cancer patients. Median OS was 38.57 months for HER2+ patients with low tumor expression of CXCL12 while median OS was 72.2 months for HER2+ patients with high tumor expression of CXCL12 (Chart 3). CXCL12 primary tumor expression was not correlated with overall survival in basal subtype (Figure 2; log rank p -value: 0.44 for OS, hazard ratio: 0.86 (0.59-1.26) (Fig. 2), or luminal B subtype breast cancer (Figure 2; log rank p -value: 0.32 for OS, hazard ratio: 0.84 (0.59-1.19) (Fig. 2).

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and down-regulation of CXCL12 was among the most significant transcriptional features in primary tumors from patients with breast cancer. CXCL12 expression in primary tumors of the breast was correlated with overall survival in patients with luminal A or HER2+ disease, with mRNA levels of CXCL12 a positive prognostic indicator for luminal A and HER2+ breast cancer patients.

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

1 tissues, we performed comparative transcriptome analysis using two independently published microarray
2 datasets^{2,3}, providing evidence here that differential expression of C-X-C motif chemokine ligand 12,
3 encoded by CXCL12, is a defining transcriptional feature of human breast cancer: in early onset-breast
4 cancer, and in patients diagnosed after age 50. CXCL12 was expressed at significantly lower levels in
5 primary tumors from patients with breast cancer as compared to normal breast tissue. Importantly, in
6 patients with luminal A or HER2+ breast cancer, expression of CXCL12 was correlated with overall
7 survival. C-X-C motif chemokine ligand 12 and the molecular processes to which it pertains to, may be
8 relevant to the initiation or progression of human breast cancer.
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Rank: 56
Probe ID: 3286602
p-value: 4.79E-16
t: 1.17E+01
B: 26.318585
Gene: CXCL12
Gene name: C-X-C motif chemokine ligand 12

Chart 1: CXCL12 is differentially expressed in the primary tumors of patients with 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.

Rank: 2619
Probe ID: 209687_at
p-value: 1.41E-10
t: 7.02
B: 13.4917829
Gene: CXCL12
Gene name: C-X-C motif chemokine ligand 12

Chart 2: CXCL12 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.

Figure 1: CXCL12 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of CXCL12 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 CXCL12 expression between normal breast tissue and primary tumors of the breast, a *p*-value, is $p < 0.0001$.

Figure 2: CXCL12 expression correlates with overall survival in patients with luminal A breast cancer or HER2+ cancer, but not in patients with basal or luminal B subtype cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=404$ patients with basal subtype breast cancer (upper left), $n=166$ patients with HER2+ breast cancer (upper right), $n=794$ patients with luminal A breast cancer (lower left), and $n=515$ patients with luminal B breast cancer (lower right), stratified into two groups, based on low or high expression of CXCL12 in patient primary tumors. The log rank p-value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on CXCL12 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Luminal A patients

Low CXCL12 expression: 108 months

High CXCL12 expression: 169.2 months

HER2+ patients

Low CXCL12 expression: 38.57 months

High CXCL12 expression: 72.2 months

Chart 3: In luminal A and in HER2+ breast cancer, median overall survival is superior in patients with high primary tumor expression of CXCL12.

The overall survival of $n=794$ luminal A breast cancer patients and $n=166$ HER2+ breast cancer patients based on stratification into low or high tumor expression of CXCL12 is listed in this chart.