Differential expression of nectin cell adhesion molecule 3 in cancers of the breast.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

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 nectin cell adhesion molecule 3, NECTIN3, when comparing primary tumors of the breast to the tissue of origin, the normal breast. NECTIN3 was also differentially expressed in the brain metastases of patients with metastatic breast cancer. NECTIN3 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 NECTIN3 in primary tumors of the breast was correlated with distant metastasis-free survival in patients with luminal B subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by PAM50 molecular subtype. NECTIN3 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, NECTIN3, nectin cell adhesion molecule 3, 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. Nectin cell adhesion molecule 3 emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE42568² and GSE52604³ 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. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ 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 NECTIN3 expression was significantly different between primary breast tumors and normal breast tissue using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of NECTIN3 mRNA expression levels with distant metastasis-free survival (DMFS) in $n=630$ patients with basal-like subtype cancer, $n=998$ patients with luminal A subtype cancer, $n=673$ patients with luminal B subtype cancer, $n=401$ patients with HER2+ cancer, and $n=63$ patients with normal-like subtype cancer.

Results

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

NECTIN3 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 nectin cell adhesion molecule 3, NECTIN3, 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, NECTIN3 ranked 45 out of 54675 total transcripts, equating to 99.9% differential expression (Chart 1). Differential expression of NECTIN3 in female breast cancer was statistically significant (Chart 1; $p=1.03E-34$).

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 NECTIN3 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, NECTIN3 ranked 6808 out of 41093 total transcripts, equating to 83.4% differential expression (Chart 2). Differential expression of NECTIN3 in the brain metastases of patients with breast cancer was statistically significant (Chart 2; $p=8.73E-04$). These data suggested that differential expression of NECTIN3 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.

NECTIN3 is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for NECTIN3 from the breast and from breast tumors to understand the magnitude and direction of NECTIN3 expression change. NECTIN3 was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of NECTIN3 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). NECTIN3 was expressed at 4.84 ± 1.20 arbitrary units (AU) in normal breast tissue, while it was expressed at 2.55 ± 0.27 AU in tumors of the breast. We calculated a mean fold change of 0.53 in NECTIN3 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

NECTIN3 expression correlates with survival outcomes in luminal B subtype human breast cancer.

We performed Kaplan-Meier survival analysis to study relationships between tumor NECTIN3 mRNA expression levels and survival outcomes in patients with breast cancer. We observed a correlation between NECTIN3 expression and distant metastasis-free survival (DMFS) in patients with luminal B subtype breast cancer which trended towards statistical significance (Figure 2; log rank p -value: 0.012 for distant metastasis-free survival, hazard ratio: 1.45 (1.08-1.95) (Fig. 2)). NECTIN3 mRNA levels were a negative prognostic indicator in luminal B subtype breast cancer patients. Median DMFS was 72.18 months for luminal B patients with low tumor expression of NECTIN3 while median DMFS was 43.2 months for luminal B patients with high tumor expression of NECTIN3 (Chart 3). NECTIN3 primary tumor expression was not correlated with distant metastasis-free survival in basal-like breast cancer (Figure 2; log rank p -value: 0.74 for DMFS, hazard ratio: 0.95 (0.71-1.28) (Fig. 2)), luminal A subtype breast cancer (Figure 2; log rank p -value: 0.79 for DMFS, hazard ratio: 1.04 (0.76-1.44) (Fig. 2)), in HER2+ breast cancer (Figure 2; log rank p -value: 0.68 for DMFS, hazard ratio: 0.93 (0.65-1.32) (Fig. 2)) or in patients with normal-like breast cancer (Figure 2; log rank p -value: 0.64 for DMFS, hazard ratio: 0.79 (0.3-2.09) (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 NECTIN3 was among the most significant transcriptional features in primary tumors from patients with breast cancer. NECTIN3 expression in primary tumors of the breast was correlated with distant metastasis-free survival in patients with luminal B subtype disease, with mRNA levels of NECTIN3 a negative prognostic indicator for luminal B 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 tissues, we performed comparative transcriptome analysis using two independently published microarray datasets^{2,3}, providing evidence here that differential expression of nectin cell adhesion molecule 3, encoded by NECTIN3, is a defining transcriptional feature of human breast cancer: in patients diagnosed

1 after age 50, and in brain metastatic tissues from patients with human breast cancer. NECTIN3 was
2 expressed at significantly lower levels in primary tumors from patients with breast cancer as compared to
3 normal breast tissue. Importantly, in patients with luminal B subtype breast cancers, expression of
4 NECTIN3 was correlated with distant metastasis-free survival. Nectin cell adhesion molecule 3 and the
5 molecular processes to which it pertains to may be relevant to the initiation or progression of human breast
6 cancer.
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

References

1. DeSantis, C.E., Ma, J., Goding Sauer, A., Newman, L.A. and Jemal, A., 2017. Breast cancer statistics, 2017, racial disparity in mortality by state. *CA: a cancer journal for clinicians*, 67(6), pp.439-448.
2. Clarke, C., Madden, S.F., Doolan, P., Aherne, S.T., Joyce, H., O'driscoll, L., Gallagher, W.M., Hennessy, B.T., Moriarty, M., Crown, J. and Kennedy, S., 2013. Correlating transcriptional networks to breast cancer survival: a large-scale coexpression analysis. *Carcinogenesis*, 34(10), pp.2300-2308.
3. Salhia, B., Kiefer, J., Ross, J.T., Metapally, R., Martinez, R.A., Johnson, K.N., DiPerna, D.M., Paquette, K.M., Jung, S., Nasser, S. and Wallstrom, G., 2014. Integrated genomic and epigenomic analysis of breast cancer brain metastasis. *Plos one*, 9(1), p.e85448.
4. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, 68(6), pp.394-424.
5. ACS Cancer Facts & Figures 2019.
<https://www.cancer.net/cancer-types/breast-cancer/metastatic/statistics>.
6. Survival Rates for Breast Cancer. <https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-survival-rates.html>
7. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*, 123(3), pp.725-731.

Rank: 45
Probe ID: 213325_at
p-value: 1.03E-34
t: 1.74E+01
B: 68.3988744
Gene: NECTIN3
Gene name: nectin cell adhesion molecule 3

Chart 1: NECTIN3 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: 6808
Probe ID: A_23_P401547
p-value: 8.73E-04
t: 3.5559351
B: -1.09908
Gene: NECTIN3
Gene name: nectin cell adhesion molecule 3

Chart 2: NECTIN3 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: NECTIN3 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of NECTIN3 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 NECTIN3 expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Figure 2: NECTIN3 expression correlates with distant metastasis-free survival in patients with luminal B subtype breast cancer, but not in patients with basal, luminal A, HER2+, or normal-like subtype cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival (DMFS) for $n=630$ patients with basal-like breast cancer (upper left), $n=401$ patients with HER2+ breast cancer (upper right), $n=998$ patients with luminal A breast cancer (middle left), $n=673$ patients with luminal B breast cancer (middle right), and $n=63$ patients with normal-like breast cancer (below) stratified into two groups, based on low or high expression of

NECTIN3 in patient primary tumors. The log rank p-value denoting statistical significance of difference in distant metastasis-free 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 NECTIN3 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 B patients

Low NECTIN3 expression: 72.18 months

High NECTIN3 expression: 43.2 months

Chart 3: In luminal B subtype breast cancer, median distant metastasis-free survival is superior in patients with low primary tumor expression of NECTIN3.

The distant metastasis-free survival of $n=673$ luminal B subtype breast cancer patients based on stratification into low or high tumor expression of NECTIN3 is listed in this chart.