Differential expression of BUB1 mitotic checkpoint serine/threonine kinase 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 BUB1 mitotic checkpoint serine/threonine kinase, BUB1, when comparing primary tumors of the breast to the tissue of origin, the normal breast. BUB1 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. BUB1 mRNA was present at significantly higher quantities in tumors of the breast as compared to normal breast tissue. Analysis of human survival data revealed that expression of BUB1 in primary tumors of the breast was correlated with distant metastasis-free survival in patients with luminal A subtype cancer. BUB1 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, BUB1, BUB1 mitotic checkpoint serine/threonine kinase, 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. BUB1 mitotic checkpoint serine/threonine kinase emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE109169² and GSE38959³ 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). 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. 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 BUB1 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 BUB1 mRNA expression levels with distant metastasis-free survival (DMFS) in $n=571$ patients with basal subtype cancer, $n=1260$ patients with luminal A subtype cancer, $n=756$ patients with luminal B subtype cancer, and $n=178$ patients with HER2+ cancer.

Results

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

BUB1 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 BUB1 mitotic checkpoint serine/threonine kinase, encoded by the BUB1 gene, 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, BUB1 ranked 187 out of 19076 total transcripts, equating to 99.0% differential expression (Chart 1). Differential expression of BUB1 in female breast cancer was statistically significant (Chart 1; $p=3.13\text{e-}13$).

Analysis of a second microarray dataset³, here in tumor cells of patients with triple negative breast cancer as compared to normal mammary gland ductal cells, again revealed significant differential expression of BUB1 in human breast cancer (Chart 2). 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 gland ductal cells, BUB1 ranked 114 out of 45015 total transcripts, equating to 99.7% differential expression (Chart 2). Differential expression of BUB1 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=1.52\text{e-}12$). These data suggested that differential expression of BUB1 was not an artifact of a single microarray dataset, nor was it associated with one type of breast cancer, rather a general feature of cancers of the breast.

BUB1 is expressed at significantly higher levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for BUB1 from the breast and from breast tumors² to understand the magnitude and direction of BUB1 expression change. BUB1 was expressed at higher levels in tumors of the breast as compared to normal breast tissue (Figure 1). Increased expression of BUB1 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). BUB1 was expressed at 5.28 ± 0.37 arbitrary units (AU) in normal breast tissue, while it was expressed at 7.01 ± 0.83 AU in tumors of the breast. We calculated a mean fold change of 1.33 in BUB1 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

BUB1 expression correlates with survival outcomes in luminal A human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate BUB1 mRNA expression levels with survival outcomes in patients with breast cancer, evaluating correlations between molecular subtype and distant metastasis-free survival. We found a statistically significant correlation between BUB1 expression and distant metastasis-free survival (DMFS) in patients with luminal A subtype breast cancer (Figure 2; log rank p -value: 0.038 for distant metastasis-free survival, hazard ratio: 1.32 (1.01-1.71) (Fig. 2)). BUB1 mRNA levels were a negative prognostic indicator in luminal A subtype breast cancer patients. Median DMFS was 236.22 months for luminal A patients with low tumor expression of BUB1 as compared to luminal A patients with high tumor expression of BUB1, who possessed median DMFS of 222.81 months (Chart 3). BUB1 primary tumor expression was not correlated with distant metastasis-free survival in basal subtype (Figure 2; log rank p -value: 0.24 for DMFS, hazard ratio: 0.83 (0.6-1.13) (Fig. 2), luminal B subtype (Figure 2; log rank p -value: 0.83 for DMFS, hazard ratio: 1.03 (0.78-1.36) (Fig. 2) or in HER2+ breast cancer (Figure 2; log rank p -value: 0.15 for DMFS, hazard ratio: 0.69 (0.42-1.15) (Fig. 2)).

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and up-regulation of BUB1 was among the most significant transcriptional features in primary tumors from patients with breast cancer. BUB1 expression in primary tumors of the breast was correlated with distant metastasis-free survival in patients with luminal A subtype cancers, with BUB1 primary tumor mRNA levels a negative prognostic indicator for luminal A 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 BUB1 mitotic checkpoint serine/threonine kinase, encoded by BUB1, is a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple negative breast cancer. BUB1 was expressed at significantly higher levels in primary tumors from patients with breast cancer, as compared to normal breast. In patients with luminal A subtype breast

cancer, primary tumor expression of BUB1 was correlated with distant metastasis-free survival. BUB1 mitotic checkpoint serine/threonine kinase, and the molecular processes to which it pertains to, may be relevant to the initiation or progression of human breast cancer.

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. Chang, J.W., Kuo, W.H., Lin, C.M., Chen, W.L., Chan, S.H., Chiu, M.F., Chang, I.S., Jiang, S.S., Tsai, F.Y., Chen, C.H. and Huang, P.H., 2018. Wild-type p53 upregulates an early onset breast cancer-associated gene GAS7 to suppress metastasis via GAS7–CYFIP1- mediated signaling pathway. *Oncogene*, 37(30), pp.4137-4150.
3. Komatsu, M., Yoshimaru, T., Matsuo, T., Kiyotani, K., Miyoshi, Y., Tanahashi, T., Rokutan, K., Yamaguchi, R., Saito, A., Imoto, S. and Miyano, S., 2013. Molecular features of triple negative breast cancer cells by genome-wide gene expression profiling analysis. *International journal of oncology*, 42(2), pp.478-506.
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: 187
Probe ID: 2570616
p-value: 3.13e-13
t: -9.74
B: 19.937385
Gene: BUB1
Gene name: BUB1 mitotic checkpoint serine/threonine kinase

Chart 1: BUB1 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: 114
Probe ID: 24624
p-value: 1.52e-12
t: -9.6486351
B: 18.445885
Gene: BUB1
Gene name: BUB1 mitotic checkpoint serine/threonine kinase

Chart 2: BUB1 is differentially expressed in the tumor cells of patients with 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.

Figure 1: BUB1 is expressed at significantly higher levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of BUB1 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 BUB1 expression between normal breast tissue and primary tumors of the breast, a *p*-value, is $p < 0.00001$.

Figure 2: BUB1 expression correlates with distant metastasis-free survival in patients with luminal A breast cancer, but not in patients with basal, luminal B or HER2+ cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival (OS) for $n=571$ patients with basal subtype breast cancer (upper left), $n=178$ patients with HER2+ breast cancer (upper right), $n=1260$ patients with luminal A breast cancer (lower left), and $n=756$ patients with luminal B breast cancer (lower right), stratified into two groups, based on low or high expression of BUB1 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 BUB1 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.

1 Low BUB1 expression: 236.22 months

2 High BUB1 expression: 222.81 months

3 **Chart 3: Median distant metastasis-free survival is superior in luminal A subtype breast cancer**
4 **patients with low primary tumor expression of BUB1.**

5 The distant metastasis-free survival of $n=1260$ luminal A subtype breast cancer patients based on
6 stratification into low or high tumor expression of BUB1 is listed in this chart.