Fibrillin-1, encoded by FBN1, is differentially expressed in lymph node metastases in human breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA 11730

Breast cancer is the most frequently diagnosed cancer in women¹. Metastasis is the major cause of death in patients diagnosed with cancer². Prognosis for patients with breast cancer worsens as the number of axillary lymph nodes with metastasis increases³. We mined published microarray and multiplexed mRNA quantitation datasets^{4,5} to discover genes associated with metastasis to the lymph nodes, an early event in breast cancer metastasis. We found significant differential expression of the gene encoding fibrillin-1, FBN1, when comparing lymph node metastases to primary breast tumors from women diagnosed with breast cancer. In primary tumors, lower expression of FBN1 correlated with worse overall survival in breast cancer patients in the lowest but not highest survival quartile. FBN1 may be of relevance to processes underlying metastasis to the lymph nodes.

Keyword: breast cancer, breast cancer metastasis, lymph node metastasis, FBN1, fibrillin-1, systems biology of breast cancer, targeted therapeutics in breast cancer.

Invasive breast cancer is diagnosed in over quarter of a million women in the United States each year¹. Metastasis is the most common cause of death in humans diagnosed with cancer². The lymphatic system, along with the blood system, are the primary routes by which tumor cells traverse the body⁶. 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^{7,8}. Patients with metastasis to distant sites are provided a 27% 5-year survival rate^{7,8}. We mined published microarray and multiplexed gene expression datasets^{4,5} to discover genes associated with lymph node metastasis in breast cancer and identified significant differential expression of FBN1. FBN1 may be of relevance to biological processes underlying breast cancer metastasis to the lymph nodes.

Methods

We used datasets GSE124648⁴ and GSE92977⁵ for this differential gene expression analysis in conjunction with GEO2R. GSE124648, a microarray dataset, was generated using Affymetrix Human Genome U133A Array technology with $n=256$ primary breast tumors from patients with breast cancer and $n=67$ lymph node metastases from patients with breast cancer; platform GPL96 was used for this analysis. GSE92977, a multiplexed mRNA quantitation dataset, was generated using NanoString nCounter Nano105 Gene Expression Custom CodeSet technology with $n=123$ primary breast tumors from patients with breast cancer and $n=24$ lymph node metastases from patients with breast cancer; platform GPL17071 was utilized for this analysis. The Benjamini and Hochberg method of p -value adjustment was used for ranking of

1 differential expression but raw p -values were used to assess statistical significance of global
2 differential expression. Log-transformation of data was auto-detected, and the NCBI generated
3 category of platform annotation was used. A statistical test was performed to evaluate whether
4 FBN1 expression was significantly between primary breast tumors and lymph node metastases
5 using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical
6 analyses of differential gene expression in human breast cancer (Version 8.4.0)(455). For
7 Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁹ for correlation of
8 FBN1 mRNA expression levels with overall survival in $n=1402$ breast cancer patients, in total, in
9 the lowest quartile and the upper quartile of survival.

14 **Results**

15 We mined published microarray and multiplexed mRNA quantitation data^{4,5} to discover
16 genes associated with metastasis of invasive breast cancer to the lymph nodes.

17 FBN1 is differentially expressed in lymph node metastases in invasive breast cancer.

18 We identified FBN1 as among the genes expressed most quantitatively differently,
19 transcriptome-wide, when comparing primary tumors of the breast to lymph node metastases in
20 women diagnosed with breast cancer⁴ (Table 1). When sorting each of the transcripts measured
21 by microarray based on significance of change in expression between lymph node metastases
22 and primary tumors of the breast, FBN1 ranked 15 out of 22283 total transcripts (Table 1).
23 Differential expression of FBN1 in lymph node metastases in human breast cancer was
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1 statistically significant (Table 1; $p=1.11\text{E-}83$).

2 Analysis of a second mRNA multiplexed quantitation dataset⁵ again revealed differential
3 expression of FBN1 in lymph node metastases when compared to primary breast tumors (Table
4 2). When sorting each of the transcripts measured by microarray based on significance of
5 change in expression between primary tumors of the breast lymph node metastases, FBN1
6 ranked 9 out of 105 transcripts measured, equating to differential expression more significant
7 than 91.4% of genes measured in this code set (Table 2). Differential expression of FBN1 in
8 lymph node metastases in human breast cancer in this second dataset was statistically significant
9 (Table 2; $p=1.33\text{E-}03$).

13
14 FBN1 is expressed at significantly lower levels in lymph node metastases when compared to
15 primary tumors of the breast.

16 We obtained exact mRNA expression levels of the differentially expressed FBN1
17 transcript to directly compare FBN1 expression between primary tumors of the breast and lymph
18 node metastases. We also performed a statistical test to evaluate whether the difference in FBN1
19 expression between primary tumors of the breast and lymph node metastases was statistically
20 significant. FBN1 was expressed at lower levels in lymph node metastases as compared to
21 primary tumors of the breast; this difference in FBN1 mRNA expression was statistically
22 significant (Figure 1; $p<0.0001$; Figure 2; $p=0.0013$). We calculated a fold change of $0.656 \pm$
23 0.254 in FBN1 mRNA expression in lymph node metastases relative to primary breast tumors
24 (Table 1).

FBN1 expression in primary breast tumors in patients with breast cancer correlates with overall survival in patients in the lowest but not highest survival quartile.

We performed Kaplan-Meier survival analysis¹⁰ using FBN1 mRNA expression in primary breast tumors coupled with paired overall survival data from each patient, in 1402 patients in total, to determine whether FBN1 tumor expression was correlated with survival outcomes in breast cancer. In the lowest survival quartile, patients whose primary tumors expressed lower levels of FBN1 possessed significantly shorter overall survival than patients with high primary tumor expression of FBN1 (Figure 3). Overall survival (OS) of patients in the low expression cohort in this sub-group was 70.44 months, while OS in patients in the high FBN1 expression cohort in this sub-group was 115 months (Table 3); this difference in OS based on FBN1 tumor expression in breast cancer was statistically significant (Figure 3; logrank p -value: 0.024; hazard ratio: 0.76 (0.6-0.97)). Kaplan-Meier analysis in all patients also demonstrated correlation between tumor expression of FBN1 and overall survival (Figure 4 and Table 4), with significant p -value ($p=0.0057$) and similar hazard ratio (0.72 (0.57-0.91) but with high false discovery rate (FDR: >50%). However, low FBN1 expression in primary tumors from patients in the highest survival quartile was not significantly correlated with worse overall survival (Figure 5 and Table 5; logrank p -value:0.57; hazard ratio (0.93 (0.73-1.19)).

Thus, we identified FBN1 as among the genes whose expression was most significantly different when comparing global gene expression of lymph node metastases from patients with breast cancer to primary tumors; FBN1 expression in lymph node metastases was significantly lower than in primary tumors, and lower expression of FBN1 in primary tumors of the breast in

1 patients from the lowest survival quartile but not the highest survival quartile was significantly
2 correlated with worse overall survival.
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4 **Discussion**

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6 The 350 kilodalton protein fibrillin-1 is a component of microfibrils of the extracellular
7 matrix containing a transforming growth factor- β binding domain (TB domain) and 47 EGF
8 domains, most of which are Ca^{2+} -binding EGF domains (cbEGF domains)¹⁰⁻¹². Mutations in
9 FBN1 cause Marfan syndrome, a connective tissue disorder affecting the heart and skeletal
10 tissues, and featuring aortic dissection and ectopia lentis^{13,14}; study of dermal fibroblasts from
11 one patient with Marfan syndrome demonstrated that only half of the mutant profibrillin was
12 secreted to the extracellular matrix and proteolytically processed; however, microfibrils from this
13 patient appeared microscopically normal¹⁵. mgR mutant mice, expressing a hypomorphic allele
14 of FBN1, produce one-quarter of the amount of fibrillin-1 as compared to wild-type mice and
15 suffer from aortic phenotypes that resemble those found in patients with Marfan syndrome¹⁶.
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17 The latent transforming growth factor β -binding protein 1, LTBP1, can interact with fibrillin-1¹⁷.
18 Fibrillin-1 can also bind integrin $\alpha\text{V}\beta 3$ through an RGD motif, an interaction that can support
19 cell attachment¹⁸. Another binding partner of fibrillin-1 is versican, which can interact with
20 fibrillin-1 peptides through its lectin domain at its carboxy-terminus in a calcium-dependent
21 fashion¹⁹.
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24 A study analyzing global gene expression profiles of patients with triple negative breast
25 cancer based on cancer subtype found that FBN1 was among the genes whose expression was
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1 relatively high in tumors expressing low levels of claudin²⁰. Methylation of the FBN1 gene has
2 been described as a potential biomarker for colorectal cancer²¹, and in ovarian cancer, fibrillin-1
3 can repress expression of E-cadherin and β -catenin, and expression of fibrillin-1 can be induced
4 by Aurora-A²². Depletion of FBN1 in OVCA429 cells *in vitro* resulted in decreased invasion,
5 transwell migration and migration speed in a scratch assay²². *In vivo*, tumors grown from OVCA
6 429 depleted of fibrillin-1 in BALB/c athymic nude mice manifested decreased tumor volume
7 and significant reduction in number of nodules in the peritoneal cavity, suggesting expression of
8 fibrillin-1 could support metastasis in ovarian cancer *in vivo*²². Higher expression of FBN1 in
9 human ovarian cancer correlated with worse patient survival²². Thus, fibrillin-1 has multiple
10 interactions partners in the extracellular matrix, its mutation results in Marfan syndrome, a
11 connective tissue disorder, and its dysregulation has been reported in multiple cancer types.

12 By mining independently published gene expression data^{4,5}, we found that the gene
13 encoding fibrillin-1, FBN1, was among the genes most differentially expressed in lymph node
14 metastases from patients with breast cancer when compared to primary tumors of the breast.
15 Lower primary tumor expression of FBN1 correlated with overall survival in patients in the
16 lowest but not highest survival quartile. FBN1 may be of relevance to processes underlying
17 spread of breast cancer to the lymph nodes.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
15	202765_s_at	1.11E-83	-26.650298	180.12622	0.656 ± 0.254	FBN1	fibrillin 1

Table 1: Differential expression of FBN1 in lymph node metastases in 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, fold change of FBN1 in lymph node metastases as compared to primary breast tumors, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
9	40	1.33E-03	-3.2713	-1.7952	FBN1

Table 2: Differential expression of FBN1 in lymph node metastases in 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: FBN1 is expressed at significantly lower levels in lymph node metastases as compared to primary tumors of the breast.

The mRNA expression level of FBN1 in primary tumors of the breast from women diagnosed with invasive breast cancer (left) and in lymph node metastases from women diagnosed with breast cancer (right) is graphically depicted with the result of a statistical test evaluating significance of difference in FBN1 expression between primary tumors and lymph node metastases, a *p*-value, listed above.

Figure 2: FBN1 is expressed at significantly lower levels in lymph node metastases as compared to primary tumors of the breast.

The mRNA expression level of FBN1 in primary tumors of the breast from women diagnosed with invasive breast cancer (left) and in lymph node metastases from women diagnosed with breast cancer (right) is graphically depicted with the result of a statistical test evaluating significance of difference in FBN1 expression between primary tumors and lymph node metastases, a *p*-value, listed above.

Figure 3: FBN1 expression in breast tumors in patients from the lowest survival quartile is significantly correlated with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of FBN1 in patient primary tumors, in the lowest quartile. 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 FBN1 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. The false discovery rate was not provided in this analysis.

Low expression cohort (months)	High expression cohort (months)
70.44	115

Table 3: FBN1 expression in breast tumors in patients from the lowest survival quartile significantly correlates with overall survival.

The overall survival of $n=1402$ breast cancer patients in the lowest quartile based on stratification into low or high tumor expression of FBN1 is listed in this chart.

Figure 4: FBN1 expression in breast tumors is correlated with overall survival in patients with breast cancer but with high false discovery rate.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of FBN1 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 FBN1 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. The false discovery rate is greater than 50%.

Low expression cohort (months)	High expression cohort (months)
67.4	118.03

Table 4: FBN1 expression in breast tumors correlates with overall survival.

The overall survival of $n=1402$ breast cancer patients based on stratification into low or high tumor expression of FBN1 is listed in this chart.

Figure 5: FBN1 expression in breast tumors in patients from the highest survival quartile is not correlated with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of FBN1 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 FBN1 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. The false discovery rate is not provided.

Low expression cohort (months)	High expression cohort (months)
95.04	118.82

Table 5: FBN1 expression in breast tumors in patients from the highest survival quartile does not significantly correlate with overall survival.

The overall survival of $n=1402$ breast cancer patients in the highest quartile based on stratification into low or high tumor expression of FBN1 is listed in this chart.