Caveolin-1, encoded by CAV1, is differentially expressed in primary tumors and lymph node metastases in human breast cancer.

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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 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 caveolin-1, CAV1, when comparing primary breast tumors to lymph node metastases from women diagnosed with breast cancer. Analysis of a separate microarray dataset⁶ revealed that CAV1 was among the genes most differentially expressed when comparing primary tumors of the breast to adjacent normal breast tissue. Low expression of CAV1, in the upper but not lower survival quartile was associated with shorter recurrence-free and overall survival. CAV1 may be of relevance to processes underlying transformation or progression of the primary tumor in human breast cancer, as well as metastasis to the lymph nodes.

Keyword: breast cancer, breast cancer metastasis, lymph node metastasis, CAV1, caveolin-1, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 Invasive breast cancer is diagnosed in over quarter of a million women in the United
2 States each year¹. Metastasis is the most common cause of death in humans diagnosed with
3 cancer². The lymphatic system is the primary route by which tumor cells traverse the body⁷.
4 While patients with localized breast cancer are provided a 99% 5-year survival rate, patients with
5 regional breast cancer, cancer that has spread to lymph nodes or nearby structures, are provided
6 an 86% 5-year survival rate^{8,9}. Patients with metastasis to distant sites are provided a 27% 5-
7 year survival rate^{8,9}. We mined published microarray gene expression datasets^{4,5} to discover
8 genes associated with the primary tumor and lymph node metastasis in breast cancer and
9 identified significant differential expression of caveolin-1, encoded by CAV1. CAV1 may be of
10 relevance to breast cancer progression or metastasis to the lymph nodes.
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15 **Methods**

16 We used datasets GSE124648⁴, GSE92977⁵, and GSE109169⁶ for this differential gene
17 expression analysis in conjunction with GEO2R. GSE124648 was generated using Affymetrix
18 Human Genome U133A Array technology with $n=256$ primary breast tumors from patients with
19 breast cancer and $n=67$ lymph node metastases from patients with breast cancer; platform GPL96
20 was used for this analysis. GSE92977, a multiplexed mRNA quantitation dataset, was generated
21 using NanoString nCounter Nano105 Gene Expression Custom CodeSet technology with $n=123$
22 primary breast tumors from patients with breast cancer and $n=24$ lymph node metastases from
23 patients with breast cancer; platform GPL17071 was utilized for this analysis. GSE109169 was
24 generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ adjacent control
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breast tissue and $n=25$ primary breast tumors from patients with breast cancer; platform GPL5175 was used for this analysis. 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 CAV1 expression was significantly between primary breast tumors and lymph node metastases, or between adjacent control tissue and primary tumors of the breast using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁹ for correlation of CAV1 mRNA expression levels with overall survival in $n=3951$ and recurrence-free survival in $n=1402$ breast cancer patients, in total, and in upper and lower survival quartiles.

Results

We mined published microarray data^{4,5,6} to discover genes associated with progression of breast cancer: between normal breast tissue and primary tumors of the breast, and between primary breast tumors and metastasis of invasive breast cancer to the lymph nodes.

CAV1 is differentially expressed in lymph node metastases in invasive breast cancer.

We identified CAV1 as among the genes expressed most quantitatively differently, transcriptome-wide, when comparing primary tumors of the breast to lymph node metastases in

1 women diagnosed with breast cancer⁴ (Table 1). When sorting each of the transcripts measured
2 by microarray based on significance of change in expression between lymph node metastases
3 and primary tumors of the breast, CAV1 ranked 165 out of 22283 total transcripts, equating to
4 differential expression more significant than 99.3% of genes measured (Table 1). Differential
5 expression of CAV1 in lymph node metastases in human breast cancer was statistically
6 significant (Table 1; $p=5.09E-43$).
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9 Analysis of a second mRNA multiplexed quantitation dataset⁵ again revealed differential
10 expression of CAV1 in lymph node metastases when compared to primary breast tumors (Table
11 2). When sorting each of the transcripts measured by microarray based on significance of
12 change in expression between primary tumors of the breast lymph node metastases, CAV1
13 ranked 24 out of 105 transcripts measured, equating to differential expression more significant
14 than 77.1% of genes measured in this code set (Table 2). Differential expression of CAV1 in
15 lymph node metastases in human breast cancer in this second dataset was statistically significant
16 (Table 2; $p=3.59E-02$).
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20 CAV1 is differentially expressed in primary tumors of the breast in invasive breast cancer.

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22 Analysis of a third microarray dataset⁶ revealed that CAV1 was also among the genes
23 most differentially expressed in primary tumors of the breast from women diagnosed with
24 invasive breast cancer when compared to adjacent normal breast tissue (Table 3). When sorting
25 each of the transcripts measured by microarray based on significance of change in expression
26 between primary tumors of the breast and adjacent, benign breast tissue, CAV1 ranked 33 out
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of 19076 transcripts measured, equating to differential expression more significant than 99.8% of genes measured (Table 3). Differential expression of CAV1 in primary tumors of the breast in human breast cancer was statistically significant (Table 3; $p=7.85E-18$).

CAV1 is expressed at significantly lower levels in lymph node metastases when compared to primary tumors of the breast.

We obtained exact mRNA expression levels of the differentially expressed CAV1 transcript to directly compare CAV1 expression between primary tumors of the breast and lymph node metastases. We also performed a statistical test to evaluate significance of difference in CAV1 expression between primary tumors of the breast and lymph node metastases was statistically significant. CAV1 was expressed at lower levels in lymph node metastases as compared to primary tumors of the breast; this difference in CAV1 mRNA expression was statistically significant (Figure 1; $p<0.0001$). We calculated a fold change of 0.8485 ± 0.1872 and 0.9024 ± 0.1697 in CAV1 mRNA expression in lymph node metastases relative to primary breast tumors (Table 1 and Table 2, respectively).

CAV1 is expressed at significantly lower levels in primary tumors of the breast when compared to adjacent benign breast tissue.

We also obtained exact mRNA expression levels of the differentially expressed CAV1 transcript to directly compare CAV1 expression between primary tumors of the breast and adjacent, benign breast tissue. We also performed a statistical test to evaluate significance of difference in CAV1 expression between breast tumors and benign breast tissue in women with invasive breast cancer. CAV1 was expressed at lower levels in primary breast tumors; this

1 difference in mRNA expression of CAV1 was statistically significant (Figure 3; $p < 0.0001$). We
2 calculated a fold change of 0.8 ± 0.1 in CAV1 mRNA expression in primary tumors of the breast
3 relative to adjacent benign breast tissue (Table 3).
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6 CAV1 expression in primary breast tumors is significantly correlated with median overall
7 survival.

8 We performed Kaplan-Meier survival analysis⁹ using CAV1 mRNA expression in
9 primary breast tumors coupled with paired overall survival data from each patient to determine
10 whether CAV1 tumor expression was correlated with survival outcomes in breast cancer.
11 Patients whose tumors expressed lower levels of CAV1 possessed shorter recurrence-free and
12 overall survival than patients with high tumor expression of CAV1 (Figure 4). Median overall
13 survival (OS) of patients in the low expression cohort was 81.6 months, while median OS in
14 patients in the high CAV1 expression cohort was 120 months (Table 4); Median recurrence-free
15 survival (RFS) of patients in the low expression cohort was 216.66 months, while median RFS in
16 patients in the high CAV1 expression cohort was 228.85 months (Table 5); difference in median
17 OS based on CAV1 tumor expression in breast cancer was statistically significant (Figure 4;
18 logrank p -value: 0.047; hazard ratio: 0.81 (0.65-1)) as was difference in RFS in breast cancer
19 based on CAV1 tumor expression (Figure 5; logrank p -value: 0.12; hazard ratio: 0.87
20 (0.78-0.97)). It is important to note, however, that for both RFS and OS Kaplan-Meier analysis
21 in all patients, the false discovery rate was unacceptably high: 20% for RFS and greater than
22 50% for OS. This could be attributable to a number of different factors.
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Similarly, median overall survival (OS) of patients in the low expression cohort was 86 months in patients in the upper survival quartile, while median OS in patients in the high CAV1 expression cohort was 169.2 months in the upper survival quartile (Table 4); Median recurrence-free survival (RFS) of patients in the low expression cohort was 44.4 months in the upper survival quartile, while median RFS in patients in the high CAV1 expression cohort was 62.88 months in the upper survival quartile (Table 5); difference in median OS based on CAV1 tumor expression in breast cancer in patients in the upper quartile was statistically significant (Figure 4; logrank p -value: 0.031; hazard ratio: 0.75 (0.57-0.98)) as was difference in RFS in breast cancer in patients in the upper quartile based on CAV1 tumor expression (Figure 5; logrank p -value: 0.00051; hazard ratio: 0.79 (0.69-0.9))

Thus, we found that caveolin-1 was among the genes whose expression was most significantly different in lymph node metastases when compared to primary tumors of the breast, and in primary tumors of the breast when compared to normal breast tissue. Caveolin-1 expression correlated with overall and recurrence-free survival; patients with low caveolin-1 expressed possessed shorter survival than those with high caveolin-1 expression.

Discussion

Mice deficient in caveolin-1 are absent of caveolae, invaginated areas of the plasma membrane, demonstrating requirement for caveolin-1 in generation of caveolae¹¹. Downstream consequences of caveolin-1 deficiency and lack of caveolae was impairment of calcium and nitric oxide signaling, resulting in defects of contraction and relaxation of heart muscle, and

1 perseveration of endothelial cell proliferation resulting in alveolar septum thickening in the
2 lungs¹¹. A study identifying high-affinity interaction partners of fatty acids in 3T3-L1 adipocytes
3 found caveolin-1 was a fatty acid binding protein¹². In endothelial cells growing in culture
4 (HMEC-1 cells), exposure to angiogenic growth factors including vascular endothelial growth
5 factor (VEGF), platelet-derived growth factor (PDGF) and hepatocyte growth factor (HGF)
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7 resulted in decreased expression of caveolin-1 in sub-confluent but not confluent cells¹³.
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9 Caveolin-1 was important for endothelial tubule formation and its expression could be observed
10 occurring prior to formation of endothelial tubules on Matrigel¹³. Consistent with a role for
11 caveolin-1 in generation of endothelial tubes, ectopic expression of caveolin-1 resulted in
12 increased formation of tubules in HMEC-1 cells¹³. A separate study found that the oncogene v-
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14 Abl could induce expression of caveolin-1, indicating that oncogenic stimuli could support
15 caveolin expression¹⁴. This appeared to be a cellular defense response against transformation as
16 opposed to supporting transformation, as expression of caveolin-1 in NIH3T3 cells impaired
17 cellular transformation in soft agar and induced apoptosis in F11 cells¹⁴. Caveolin-1 can also
18 function as a sort of an adaptor, linking together $\beta 1$ integrins to the Fyn kinase; integrin ligation
19 by Fyn results in recruitment of Shc and activation of the extracellular signal-related kinase
20 (ERK) important for cell cycle progression, suggesting that caveolin-1 is required for induction
21 anchorage-dependent cell growth through integrin signaling¹⁵. Thus, among its many roles,
22 caveolin-1 functions as an integral component of plasma membrane microdomain structures
23 known as caveolae, is a fatty-acid binding protein, can influence angiogenesis and endothelial
24 tubule formation, and is described as critical for anchorage-dependent cell growth.
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Multiple studies have previously implicated caveolin-1 in the biology of breast cancer, including other solid tumors such as sarcoma. While expressed at relatively high levels in fibromatoses, leiomyomas, hemangiomas and lipomas compared to mesenchymal tissues, caveolin-1 expression was decreased in 4 of 6 liposarcomas, 17 of 20 leiomyosarcomas, 16 of 16 myxoid/round cell/pleomorphic liposarcomas, 5 of 8 angiosarcomas, 15 of 18 malignant fibrous histiocytomas, and 8 of 8 synovial sarcomas. Importantly, ectopic expression of caveolin-1 in HT-1080 fibrosarcoma cells resulted in marked reduction in colony formation *in vitro*¹⁶. One study found mutation of the CAV1 gene in 16% of human breast cancers surveyed¹⁷. The resulting mutation, P132L, has oncogenic properties as its expression can transform NIH 3T3 cells *in vitro*¹⁷. P132L functions in a dominant-negative manner, preventing proper sub-cellular localization of wild-type caveolin-1 to caveolae¹⁸. Critically, specific genetic deletion of CAV1 in mice resulted in intraductal hyperplasia in the mammary gland, suggesting that caveolin-1 can function to prevent mammary epithelial cell hyperplasia *in vivo*¹⁸. Caveolin-1 is expressed at lower levels in highly metastatic 4T1.2 and 4T1.13 tumors as compared to non-metastatic tumors, which is consistent with our analysis of published microarray data from human breast cancers¹⁹. Similarly, high expression of caveolin-1 resulted in decreased metastasis to the lungs and to the bones when expressed in 4T1.2 cells grafted in mice¹⁹. Loss of caveolin-1 expression in the tumor stroma was associated with disease recurrence, tumor stage, and consistent with our analysis of published microarray data, metastasis to the lymph nodes²⁰. In breast cancer patients treated with tamoxifen, loss of caveolin-1 expression in the stroma predicted worse patient outcome suggestive of tamoxifen resistance²⁰.

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2 Of critical importance, and again consistent with our analysis of microarray data from primary
3 tumors, loss of caveolin-1 expression in the stroma predicted 11.5-fold reduction in the 5-year
4 progression-free survival rate for breast cancer patients considered “lymph-node positive” or
5 with metastasis to the lymph nodes²⁰. Clearly, multiple studies support our analysis of published
6 microarray data based on analysis of multiple microarray and multiplexed gene expression
7 datasets^{4,5,6} that reduced or lost expression of caveolin-1 is associated with metastasis to the
8 lymph nodes in human breast cancer and can predict or is correlated with worse patient
9 outcomes.
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13 By mining independently published gene expression data^{4,5,6}, we found that the gene
14 encoding caveolin-1, CAV1, was among the genes most differentially expressed in human breast
15 cancer, both in lymph node metastases when compared to primary tumors of the breast as well as
16 in primary tumors of the breast as compared to adjacent benign breast tissue. CAV1 appears to
17 be expressed in a gradient-like fashion in invasive breast cancer, and may be of relevance to
18 processes underlying progression of breast cancer from transformation to spread of breast cancer
19 to the lymph nodes.
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References

1. DeSantis, C.E., Ma, J., Gaudet, M.M., Newman, L.A., Miller, K.D., Goding Sauer, A., Jemal, A. and Siegel, R.L., 2019. Breast cancer statistics, 2019. *CA: a cancer journal for clinicians*, 69(6), pp.438-451.
2. Mehlen, P. and Puisieux, A., 2006. Metastasis: a question of life or death. *Nature reviews cancer*, 6(6), pp.449-458.
3. Fisher, B., Bauer, M., Wickerham, D.L., Redmond, C.K., Fisher, E.R., Cruz, A.B., Foster, R., Gardner, B., Lerner, H., Margolese, R. and Poisson, R., 1983. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP update. *Cancer*, 52(9), pp.1551-1557.
4. Sinn, B.V., Fu, C., Lau, R., Litton, J., Tsai, T.H., Murthy, R., Tam, A., Andreopoulou, E., Gong, Y., Murthy, R. and Gould, R., 2019. SET ER/PR: a robust 18-gene predictor for sensitivity to endocrine therapy for metastatic breast cancer. *NPJ breast cancer*, 5(1), pp.1-8.
5. Cejalvo, J.M., de Dueñas, E.M., Galván, P., García-Recio, S., Gasión, O.B., Paré, L., Antolin, S., Martinello, R., Blancas, I., Adamo, B. and Guerrero-Zotano, Á., 2017. Intrinsic subtypes and gene expression profiles in primary and metastatic breast cancer. *Cancer research*, 77(9), pp.2213-2221.
6. 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.
7. Rahman, M. and Mohammed, S., 2015. Breast cancer metastasis and the lymphatic system. *Oncology letters*, 10(3), pp.1233-1239.
8. ACS Cancer Facts & Figures 2019. <https://www.cancer.net/cancer-types/breast-cancer-metastatic/statistics>.
9. Survival Rates for Breast Cancer. <https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-survival-rates.html>
10. 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.

11. Drab, M., Verkade, P., Elger, M., Kasper, M., Lohn, M., Lauterbach, B., Menne, J., Lindschau, C., Mende, F., Luft, F.C. and Schedl, A., 2001. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *science*, 293(5539), pp.2449-2452.
12. Trigatti, B.L., Anderson, R.G. and Gerber, G.E., 1999. Identification of caveolin-1 as a fatty acid binding protein. *Biochemical and biophysical research communications*, 255(1), pp. 34-39.
13. Liu, J., Wang, X.B., Park, D.S. and Lisanti, M.P., 2002. Caveolin-1 expression enhances endothelial capillary tubule formation. *Journal of Biological Chemistry*, 277(12), pp. 10661-10668.
14. Engelman, J.A., Wykoff, C.C., Yasuhara, S., Song, K.S., Okamoto, T. and Lisanti, M.P., 1997. Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. *Journal of Biological Chemistry*, 272(26), pp.16374-16381.
15. Wary, K.K., Mariotti, A., Zurzolo, C. and Giancotti, F.G., 1998. A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell*, 94(5), pp.625-634.
16. Wiechen, K., Sers, C., AgoulNIK, A., Arlt, K., Dietel, M., Schlag, P.M. and Schneider, U., 2001. Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. *The American journal of pathology*, 158(3), pp.833-839.
17. Hayashi, K., Matsuda, S., Machida, K., Yamamoto, T., Fukuda, Y., Nimura, Y., Hayakawa, T. and Hamaguchi, M., 2001. Invasion activating caveolin-1 mutation in human scirrhous breast cancers. *Cancer research*, 61(6), pp.2361-2364.
18. Lee, H., Park, D.S., Razani, B., Russell, R.G., Pestell, R.G. and Lisanti, M.P., 2002. Caveolin-1 mutations (P132L and null) and the pathogenesis of breast cancer: caveolin-1 (P132L) behaves in a dominant-negative manner and caveolin-1 (-/-) null mice show mammary epithelial cell hyperplasia. *The American journal of pathology*, 161(4), pp. 1357-1369.
19. Sloan, E.K., Stanley, K.L. and Anderson, R.L., 2004. Caveolin-1 inhibits breast cancer growth and metastasis. *Oncogene*, 23(47), pp.7893-7897.
20. Witkiewicz, A.K., Dasgupta, A., Sotgia, F., Mercier, I., Pestell, R.G., Sabel, M., Kleer, C.G., Brody, J.R. and Lisanti, M.P., 2009. An absence of stromal caveolin-1 expression predicts early tumor recurrence and poor clinical outcome in human breast cancers. *The American journal of pathology*, 174(6), pp.2023-2034.

Rank	ID	p-value	t	B	FC	Gene	Gene name
165	212097_at	5.09E-43	-16.036577	87.06851	0.8485 ± 0.1872	CAV1	caveolin 1

Table 1: Differential expression of CAV1 in lymph node metastases in breast cancer.

Rank of differential expression, 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 CAV1 in lymph node metastases as compared to primary breast tumors, and gene are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene
24	11	3.59E-02	-2.1176	-4.7283	0.9024 ± 0.1697	CAV1

Table 2: Differential expression of CAV1 in lymph node metastases in breast cancer.

Rank of differential expression, 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 CAV1 in lymph node metastases as compared to primary breast tumors, and gene are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
33	3020302	7.85E-18	-1.3E+01	30.348134	0.8 ± 0.1	CAV1	caveolin 1

Table 3: Differential expression of CAV1 in primary tumors of the breast in breast cancer.

Rank of differential expression, 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 CAV1 in primary tumors of the breast as compared to adjacent benign breast tissue, and gene are listed in this chart.

Figure 1: CAV1 is expressed at significantly lower levels in lymph node metastases as compared to primary tumors of the breast.

The mRNA expression level of CAV1 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 CAV1 expression between primary tumors and lymph node metastases, a *p*-value, listed above.

Figure 2: CAV1 is expressed at significantly lower levels in lymph node metastases as compared to primary tumors of the breast.

The mRNA expression level of CAV1 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 CAV1 expression between primary tumors and lymph node metastases, a *p*-value, listed above.

Figure 3: CAV1 is expressed at significantly lower levels in primary tumors of the breast as compared to benign adjacent breast tissue.

The mRNA expression level of CAV1 in benign, tumor-adjacent 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 CAV1 expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Figure 4: CAV1 expression in breast tumors is significantly correlated with overall survival in patients with breast cancer, as a whole and in patients in the upper survival quartile, but with a high false-discovery rate.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=3951$ total patients stratified into two groups, based on low or high expression of CAV1 in patient primary tumors. Analysis is performed for all patients (A), patients in the lower survival quartile (B), and patients in the upper survival quartile (C). 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 CAV1 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 for RFS in all patients was greater than 50%.

	Low expression cohort (months)	High expression cohort (months)
All patients	81.6	120
Upper quartile	86	169.2

Table 4: CAV1 expression in breast tumors significantly correlates with patient survival.

The median overall survival of $n=1402$ breast cancer patients based on stratification into low or high tumor expression of LHFP is listed in this chart, in two patient populations: encompassing all patients, or encompassing only the upper survival quartile.

Figure 5: CAV1 expression in breast tumors is significantly correlated with recurrence-free survival in patients with breast cancer, as a whole and in patients in the upper survival quartile, but with a high false-discovery rate.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival (RFS) for $n=3951$ total patients stratified into two groups, based on low or high expression of CAV1 in patient primary tumors. Analysis is performed for all patients (A), patients in the lower survival quartile (B), and patients in the upper survival quartile (C). 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 CAV1 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 for RFS in all patients is 20%.

	Low expression cohort (months)	High expression cohort (months)
All patients	216.66	228.85
Upper quartile	44.4	62.88

Table 5: CAV1 expression in breast tumors significantly correlates with patient survival.

The median overall survival of $n=1402$ breast cancer patients based on stratification into low or high tumor expression of LHFP is listed in this chart, in two patient populations: encompassing all patients, or encompassing only the upper survival quartile.