Secreted protein acidic and cysteine rich (SPARC) is differentially expressed in metastatic breast cancer, both in metastases to the brain and to the lymph nodes.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data^{4,5} to compare primary and metastatic tumor transcriptomes to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that secreted protein acidic and cysteine rich, encoded by SPARC, also known as osteonectin, was among the genes whose expression was most different in the metastatic tumor tissues of patients with metastatic breast cancer, both in metastases to brain and to the lymph nodes when compared to primary tumors of the breast or normal breast tissue, respectively. We observed significant down-regulation of SPARC in metastasis to the brain. Molecular functions and down-regulation of secreted protein acidic and cysteine rich, a protein whose down-regulation appears to be critical for survival of ovarian cancer cells, may be important for metastasis of primary tumor-derived cancer cells to the lymph nodes and to the brain in humans with metastatic breast cancer and suggests some level of common origin for metastases that reside in the lymph nodes and colonize the brain.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, SPARC, secreted protein acidic and cysteine rich, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer². This alarmingly high frequency of CNS metastasis events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. We performed a global comparative analysis of primary and metastatic tumors in patients with metastatic breast cancer^{4,5}. We discovered transcriptome-wide differential expression of SPARC in brain and lymph node metastases of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE124648⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. GSE124648, a microarray dataset, was generated using Affymetrix Human Genome U133A Array technology with $n=10$ normal breast tissues and $n=44$ lymph node metastases from patients with breast cancer; platform GPL96 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 SPARC gene expression was significantly between primary breast tumors and brain metastases in humans with breast cancer 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).

Results

We performed global comparative transcriptome analysis of the primary and metastatic tumor tissues of patients with brain and lymph node metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

SPARC is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified secreted protein acidic and cysteine rich, encoded by SPARC, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁴ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to the primary tumors of patients with brain metastatic breast cancer, SPARC ranked 11 out of 22277 total transcripts (Table 1), equating to 99.95% differential expression. Differential expression of SPARC in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=7.99E-07$).

We asked whether we could validate differential expression of SPARC was specific to brain metastasis or if we could observe differential expression of SPARC in lymph node metastasis. Comparison of global gene expression profiles of 10 normal breast tissues to 44 lymph node metastases⁵ revealed that SPARC was among the genes whose expression was most significantly different transcriptome-wide between the breast and lymph node metastases in patients with metastatic breast cancer (Table 2).

When sorting each of the genes expressed in lymph node metastases based on significance of difference in expression as compared to normal breast tissues, SPARC ranked 390 out of 22283 total transcripts (Table 2), equating to 98.2% differential expression. Differential expression of SPARC in lymph node metastases from patients with metastatic breast cancer was statistically significant (Table 2; $p=9.51E-10$).

SPARC is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for SPARC, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and direction of change in SPARC expression in brain metastatic tissues. SPARC was expressed at lower levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.84 ± 0.07 in SPARC expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified SPARC as a differentially expressed gene in metastatic breast cancer, both in brain metastases and in lymph node metastases when compared to tissues of the breast, transformed and benign; SPARC was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast.

Discussion

We provided evidence here that secreted protein acidic and cysteine rich, encoded by SPARC is among the genes whose expression is most different in the brain and lymph node metastases of patients with metastatic breast cancer. SPARC, also known as osteonectin, was originally described to possess bone-specific expression and to link apatite crystals to collagen I⁶. Later, down-regulation of SPARC was described as important for ovarian carcinomas, as its expression induced apoptosis of ovarian cancer cells⁷. SPARC^{8,9} may be of relevance to any number of steps required for metastasis to the lymph nodes and to the brain, and differential expression of SPARC in both metastasis to the lymph nodes and the brain suggests some level of common origin for metastases that reside in the lymph nodes and colonize the brain.

References

1. Lin, N.U., Amiri-Kordestani, L., Palmieri, D., Liewehr, D.J. and Steeg, P.S., 2013. CNS metastases in breast cancer: old challenge, new frontiers.
2. Bendell, J.C., Domchek, S.M., Burstein, H.J., Harris, L., Younger, J., Kuter, I., Bunnell, C., Rue, M., Gelman, R. and Winer, E., 2003. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer*, 97(12), pp.2972-2977.
3. Tsukada, Y., Fouad, A., Pickren, J.W. and Lane, W.W., 1983. Central nervous system metastasis from breast carcinoma autopsy study. *Cancer*, 52(12), pp.2349-2354.
4. Iwamoto, T., Niikura, N., Ogiya, R., Yasojima, H., Watanabe, K.I., Kanbayashi, C., Tsuneizumi, M., Matsui, A., Fujisawa, T., Iwasa, T. and Shien, T., 2019. Distinct gene expression profiles between primary breast cancers and brain metastases from pair-matched samples. *Scientific reports*, 9(1), pp. 1-8.
5. 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.
6. Termine, J.D., Kleinman, H.K., Whitson, S.W., Conn, K.M., McGarvey, M.L. and Martin, G.R., 1981. Osteonectin, a bone-specific protein linking mineral to collagen. *Cell*, 26(1), pp.99-105.
7. Yiu, G.K., Chan, W.Y., Ng, S.W., Chan, P.S., Cheung, K.K., Berkowitz, R.S. and Mok, S.C., 2001. SPARC (secreted protein acidic and rich in cysteine) induces apoptosis in ovarian cancer cells. *The American journal of pathology*, 159(2), pp.609-622.
8. Goldblum, S.E., Ding, X., Funk, S.E. and Sage, E.H., 1994. SPARC (secreted protein acidic and rich in cysteine) regulates endothelial cell shape and barrier function. *Proceedings of the National Academy of Sciences*, 91(8), pp.3448-3452.
9. Lane, T.F. and Sage, E.H., 1994. The biology of SPARC, a protein that modulates cell-matrix interactions. *The FASEB Journal*, 8(2), pp.163-173.

Rank	ID	p-value	t	B	FC	Gene	Gene name
11	200665_s_at	7.99E-07	-6.0057156	5.539	0.84 ± 0.07	SPARC	secreted protein acidic and cysteine rich

Table 1: SPARC is differentially expressed in brain metastases in metastatic breast cancer when compared to primary tumors of the breast.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change (FC) of SPARC expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
390	212667_at	9.51E-10	7.352365	12.00454	SPARC	secreted protein acidic and cysteine rich

Table 2: SPARC is differentially expressed in lymph node metastases in metastatic breast cancer when compared to normal breast tissues.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: SPARC is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of SPARC in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean SPARC levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above.