LINC00968 is differentially expressed in brain metastatic breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Breast cancer patients diagnosed with metastasis to the brain¹⁻³ are presented limited treatment options^{4,5}. We mined published microarray data^{6,7} to discover genes associated with brain metastasis in human breast cancer at the transcriptome-level and in an unbiased fashion. We describe here the differential expression of the long intergenic non-coding RNA LINC00968 in the brain metastatic tissues and primary tumors of women with breast cancer. LINC00968 transcript was present at significantly reduced quantities at in brain metastases and in primary tumors of the breast as compared to untransformed breast tissues. Molecular functions and down-regulation of LINC00968, a non-coding RNA that has not previously been described in relation to central nervous system metastasis in human breast cancer represents a feature of the transcriptional fingerprint of brain metastatic breast cancer in humans and may be relevant to pathways underlying invasion and colonization of the brain.

Keywords: breast cancer, LINC00968, long intergenic non-coding RNA 00968, brain metastasis, central nervous system metastasis, systems biology of breast cancer, targeted therapeutics in breast cancer.

A study of patients treated with treated with trastuzumab, a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2), reported a rate of metastasis to the central nervous system of 34%². The severity of disease and limited treatment options demand an enhanced understanding of the fundamental transcriptional nature of metastasis to the brain in patients with breast cancer. We mined published microarray data^{6,7} generated using the primary tumors and brain metastatic tissues of patients with human breast cancer to identify genes whose expression was most quantitatively different in brain metastatic tissues. These analyses revealed significant differential expression of LINC00968 in metastases to the brain in patients with human breast cancer.

Methods

We used datasets GSE52604⁶ and GSE42568⁷ and for this differential gene expression analysis of brain metastasis in human breast cancer in conjunction with GEO2R. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer; analysis was performed using platform GPL6480. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=17$ primary tumors of the breast and $n=104$ brain metastases from patients with breast cancer; analysis was performed using GPL570. 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 LINC00968 gene expression was significantly between primary breast tumors and normal breast tissues or brain metastases and normal breast tissues 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 comparative transcriptome analysis of primary and metastatic tumor tissues from patients with breast cancer using published microarray data^{6,7} to describe the transcriptional landscape of brain metastasis in human breast cancer and for the identification of potential therapeutic targets.

LINC00968 is differentially expressed in the brain metastases of patients with metastatic breast cancer when compared to tissues of the breast, both benign and transformed.

We identified the gene encoding the long intergenic non-coding RNA LINC00968 as among the genes whose expression was most significantly different in the brain metastatic tissues of patients with breast cancer as compared to normal breast tissues. When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to benign breast tissue, LINC00968 ranked 24 out of 41093 total transcripts (Table 1), equating to 99.94% differential expression. Differential expression of LINC00968 in metastases to the brain was statistically significant (Table 1; $p=7.46E-17$).

We mined a second microarray dataset to validate differential expression of LINC00968 in brain metastatic breast cancer. We again identified LINC00968 as among the genes whose expression was most different in the brain metastatic tissues of patients with breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to primary tumors of the breast, in this dataset, LINC00968 ranked 220 out of 54675 total transcripts (Table 2), equating to 99.6% differential expression. Differential expression of LINC00968 in metastases to the brain was again statistically significant (Table 2; $p=3.59E-26$).

LINC00968 is expressed at significantly lower levels in metastases to the brain as compared to primary tumors of the breast.

We obtained exact mRNA expression levels for LINC00968 from brain metastases of patients with breast cancer and from normal breast tissue to evaluate direction and statistical significance of change in LINC00968 mRNA expression in metastasis to the brain in human metastatic breast cancer. LINC00968 was expressed at lower levels in metastasis to the brain as compared to normal breast tissue (Figure 1), and this difference was statistically significant (Figure 1; $p < 0.0001$).

We also obtained exact mRNA expression levels for LINC00968 from primary tumors of the breast and from brain metastases of patients with breast cancer to evaluate magnitude and direction of change in LINC00968 mRNA expression in metastasis to the brain in human breast cancer and to determine whether this change was statistically significant. LINC00968 was expressed at lower levels in metastasis to the brain as compared to normal breast tissue (Figure 2), and this difference was statistically significant (Figure 2; $p < 0.0001$). We calculated a mean fold change of 0.44 ± 0.06 in LINC00968 transcript levels when comparing primary tumors of the breast to normal breast tissues (Table 2; $p = 0.0001$).

Thus, through blinded, systems-level analysis of published microarray datasets^{6,7}, we found that LINC00968 was among the genes whose expression was most significantly different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast, and that LINC00968 expression was significantly lower in brain metastatic tissues as compared to normal breast tissues and primary tumors of the breast.

Discussion

We provided evidence here that in tumor tissues isolated from humans, the long intergenic non-coding RNA LINC00968 was among the genes most differentially expressed in metastases to the brain as compared to either normal breast tissue or primary tumors of the breast. LINC00968 was expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast. LINC00968, despite possessing oncogenic qualities in studies of non-small cell lung cancer⁸ and osteosarcoma⁹ *in vitro*, has been reported to display tumor suppressive qualities in studies of breast cancer cell lines¹⁰ (e.g., impairment of cellular proliferation and angiogenesis *in vitro*) and may be of relevance to the processes which control metastasis of tumor cells from the breast to 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. Robson, M., Im, S.A., Senkus, E., Xu, B., Domchek, S.M., Masuda, N., Delaloge, S., Li, W., Tung, N., Armstrong, A. and Wu, W., 2017. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *New England Journal of Medicine*, 377(6), pp.523-533.
5. Turner, N.C., Ro, J., André, F., Loi, S., Verma, S., Iwata, H., Harbeck, N., Loibl, S., Huang Bartlett, C., Zhang, K. and Giorgetti, C., 2015. Palbociclib in hormone-receptor-positive advanced breast cancer. *New England Journal of Medicine*, 373(3), pp.209-219.
6. 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.
7. 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.
8. Wang, Y., Zhou, J., Xu, Y.J. and Hu, H.B., 2018. Long non-coding RNA LINC00968 acts as oncogene in NSCLC by activating the Wnt signaling pathway. *Journal of cellular physiology*, 233(4), pp.3397-3406.
9. Liu, G., Yuan, D., Sun, P., Liu, W., Wu, P.F., Liu, H. and Yu, G.Y., 2018. LINC00968 functions as an oncogene in osteosarcoma by activating the PI3K/AKT/mTOR signaling. *Journal of cellular physiology*, 233(11), pp.8639-8647.
10. Sun, X., Huang, T., Zhang, C., Zhang, S., Wang, Y., Zhang, Q. and Liu, Z., 2019. Long non-coding RNA LINC00968 reduces cell proliferation and migration and angiogenesis in breast cancer through up-regulation of PROX1 by reducing hsa-miR-423-5p. *Cell Cycle*, 18(16), pp.1908-1924.

Rank	ID	p-value	t	B	Gene	Gene name
24	A_24_P862083	7.46E-17	-12.747683	27.979643	LINC00968	long intergenic non-protein coding RNA 968

Table 1: Differential expression of LINC00968 in brain metastatic tissues as compared to normal breast tissues in patients with breast cancer.

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 LINC00968 expression between the brain metastases of patients with breast cancer and normal tissues of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
220	243813_at	3.59E-26	-1.36E+01	48.9694421	0.44 ± 0.06	LINC00968	long intergenic non-protein coding RNA 968

Table 2: Differential expression of LINC00968 in primary tumors of the breast as 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: LINC00968 is expressed at significantly lower levels in brain metastases in patients with metastatic breast cancer.

The mRNA expression level of LINC00968 in benign breast tissue (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean LINC00968 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal tissues of the breast, a *p*-value, listed above.

Figure 2: LINC00968 is expressed at significantly lower levels in primary tumors of the breast in patients with breast cancer.

The mRNA expression level of LINC00968 in benign breast tissue (left) and in the primary tumors of breast cancer patients (right) is graphically represented here with mean LINC00968 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal tissues of the breast, a *p*-value, listed above.