Coding and non-coding transcripts produced at the TBX5 locus are differentially expressed in brain metastatic breast cancer.

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Control of gene expression includes regulation by *trans*-acting transcription factors and *cis*-acting epigenetic regulation by chemical modification of histones and the DNA^{1,2}. Brain metastases are a clinical problem in patients with breast cancer³⁻⁵. We mined published microarray data^{6,7} to discover genes associated with brain metastasis in patients with brain metastatic breast cancer. We found that the gene encoding the T-box transcription factor TBX5 was among the genes most differentially expressed in the brain metastases of patients with brain metastatic breast cancer. Differential expression of anti-sense transcription at the TBX5 locus in brain metastases in patients with metastatic breast cancer was also observed. TBX5 may be of relevance to the biology underlying metastasis to the brain.

Keywords: breast cancer, brain metastases, central nervous system metastases, TBX5, TBX5-AS1, 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 cancer⁴. 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. We performed a global comparative analysis of transcription in the brain metastases of patients with brain metastatic breast cancer^{6,7}. We discovered significant differential expression and down-regulation of TBX5, a T-box-containing transcription factor in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁶ and GSE52604⁷ 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. 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 performed using platform GPL6480. 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 TBX5 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 metastatic breast cancer using published microarray data^{6,7} for the discovery of novel therapeutic targets.

TBX5 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the transcription factor TBX5 as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁶ (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, TBX5 ranked 169 out of 22297 total transcripts (Table 1), equating to 99.2% differential expression. Differential expression of TBX5 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=1.12E-03$).

Cross-validation of differentially expressed genes across datasets in cancer can be difficult and more challenging than when cross-validating across organs and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked whether we could validate differential expression of TBX5 when comparing brain metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the breast. Comparison of global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁷ revealed that TBX5 was among the genes whose expression was most significantly different transcriptome-wide between the breast and brain metastases in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal tissues of the breast, TBX5 ranked 29 out of 41093 total

transcripts (Table 2), equating to 99.9% differential expression. Differential expression of TBX5 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=2.27E-16$).

We also observed differential expression of a non-coding, anti-sense RNA produced at the TBX5 locus, TBX5-AS1, in brain metastases from patients with breast cancer when compared to normal breast tissue (Table 3). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal breast tissue, TBX5-AS1 ranked 5 out of 41093 total transcripts (Table 3), equating to 99.99% differential expression. Differential expression of TBX5-AS1 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 3; $p=3.96E-25$).

TBX5 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for TBX5, 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 TBX5 expression in brain metastatic tissues. TBX5 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; $p=0.0005$). We calculated a mean fold change of 0.807 ± 0.147 in TBX5 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^{6,7} in an unbiased and systematic fashion, we identified the transcription factor TBX5 as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to primary tumors of the breast and to the normal breast; TBX5 was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast.

Discussion

The T-box-containing transcription factor TBX5 is mutated in Holt-Oram syndrome, a genetically transmitted developmental disorder resulting in abnormalities of the heart and the limbs⁸. T-box genes⁹ are transcription factors related to the transcription factor defective in the mutant mouse T¹⁰, also known as Brachyury, deficient in mesoderm generation, and related to the *Drosophila* optomotor blind¹¹. Tbx5 is expressed in human embryos during development, and modeling these mutations in mice by haploinsufficiency of the murine homologue of human Tbx5, TbxS, results in similar abnormalities of the heart and forelimbs¹²; thus, Tbx5 is essential for the development of the heart and the limbs in mammals. Tbx5 also has developmental functions in the central nervous system; Tbx5 is important for guiding formation of retinal projections to the tectum, as Tbx5 misexpression results in disruption of projections from the axons of retinal ganglion¹³. In the development of the limbs, Tbx5 interacts with Wnt2b¹⁴, which we recently demonstrated is present at higher quantities at the mRNA level in the primary tumors of breast cancer patients treated with trastuzumab¹⁵; the frequency of central nervous system metastases in breast cancer patients treated with trastuzumab, in one report, was 34%⁴.

We report here that the transcription factor TBX5, which controls development of the circuitry providing visual sensory input from the retina to the brain, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. TBX5 and its genomic targets, potentially interacting with certain treatment regimens, may be of relevance to the biology underlying metastasis to the brain in patients with brain metastatic breast cancer, a disease with limited treatment options.

References

1. Herdegen, T. and Leah, J.D., 1998. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Research Reviews*, 28(3), pp.370-490.
2. Grewal, S.I. and Moazed, D., 2003. Heterochromatin and epigenetic control of gene expression. *Science*, 301(5634), pp.798-802.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. Li, Q.Y., Newbury-Ecob, R.A., Terrett, J.A., Wilson, D.I., Curtis, A.R., Yi, C.H., Gebuhr, T., Bullen, P.J., Robson, S.C., Strachan, T. and Bonnet, D., 1997. Holt-Oram syndrome is caused by mutations in TBX5, a member of the Brachyury (T) gene family. *Nature genetics*, 15(1), pp.21-29.
9. Wilson, V. and Conlon, F.L., 2002. The T-box family. *Genome biology*, 3(6), pp.1-7.
10. Herrmann, B.G., Labeit, S., Poustka, A., King, T.R. and Lehrach, H., 1990. Cloning of the T gene required in mesoderm formation in the mouse. *Nature*, 343(6259), pp.617-622.
11. Pflugfelder, G.O., Roth, H. and Poeck, B., 1992. A homology domain shared between Drosophila optomotor-blind and mouse Brachyury is involved in DNA binding. *Biochemical and biophysical research communications*, 186(2), pp.918-925.

- 1 12.Bruneau, B.G., Nemer, G., Schmitt, J.P., Charron, F., Robitaille, L., Caron, S., Conner, D.A., Gessler,
2 M., Nemer, M., Seidman, C.E. and Seidman, J.G., 2001. A murine model of Holt-Oram syndrome
3 defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. *Cell*, 106(6), pp.
4 709-721.
- 5 13.Koshiba-Takeuchi, K., Takeuchi, J.K., Matsumoto, K., Momose, T., Uno, K., Hoepker, V., Ogura, K.,
6 Takahashi, N., Nakamura, H., Yasuda, K. and Ogura, T., 2000. Tbx5 and the retinotectum
7 projection. *Science*, 287(5450), pp.134-137.
- 8 14.Ng, J.K., Kawakami, Y., Büscher, D., Raya, Á., Itoh, T., Koth, C.M., Esteban, C.R., Rodríguez-León,
9 J., Garrity, D.M., Fishman, M.C. and Belmonte, J.C.I., 2002. The limb identity gene Tbx5 promotes
10 limb initiation by interacting with Wnt2b and Fgf10. *Development*, 129(22), pp.5161-5170.
- 11 15.Mamoor, Shahan. "Trastuzumab administration in patients with breast cancer is associated with
12 increased primary tumor expression of Wnt2 and Wnt10b." (2020).

Rank	ID	p-value	t	B	FC	Gene	Gene name
169	207155_at	1.12E-03	-3.5545796	-0.8356	0.807 ± 0.147	TBX5	T-box 5

Table 1: TBX5 is differentially expressed in brain metastases in brain metastatic 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 TBX5 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
29	A_24_P30557	2.27E-16	-12.3642315	26.917754	TBX5	T-box 5

Table 2: TBX5 is differentially expressed in brain metastases in brain metastatic 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, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
5	A_32_P158875	3.96E-25	-20.5747458	45.638899	TBX5-AS1	TBX5 antisense RNA 1

Table 3: An anti-sense transcript produced at the TBX5 locus is differentially expressed in brain metastases in brain metastatic 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, gene and gene name are listed in this chart.

Figure 1: TBX5 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

The mRNA expression level of TBX5 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 TBX5 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.

Figure 2: TBX5 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to normal breast tissue.

The mRNA expression level of TBX5 in normal breast tissue (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean TBX5 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.

Figure 3: TBX5-AS1 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to normal breast tissue.

The mRNA expression level of TBX5-AS1 in normal breast tissue (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean TBX5-AS1 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.