Tyrosine kinase BMX is differentially expressed in the brain metastases of patients with metastatic breast cancer.

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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 tyrosine kinase BMX was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to normal breast tissues. BMX mRNA was present at significantly reduced quantities in brain metastatic tissues as compared to normal and transformed breast tissues. Down-regulation of signals transduced by BMX may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, BMX, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in patients treated with trastuzumab for breast cancer². More recently, the NEfERT-T clinical trial⁶ which compared administration of either neratinib or trastuzumab in conjunction with paclitaxel demonstrated that in a randomized, controlled setting, in breast cancer patients treated with neratinib, not only was the incidence of central nervous system recurrence significantly lower, the time to central nervous system metastasis was significantly delayed as compared to patients administered trastuzumab. The alarmingly high rate of central nervous system metastasis described, as well as data, both anecdotal² and from a randomized, controlled setting⁶ illustrating that treatment with trastuzumab may be associated with these 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 the benign breast, primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and decreased expression of the tyrosine kinase BMX in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE26338⁵ and for this global differential gene expression analysis of brain metastatic 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 performed using platform GPL6480. GSE26338 was generated using Human 1A Microarray G4110A technology with n=11 primary breast tumors and n=3 brain metastases from patients with breast cancer, analysis performed using platform GPL885. 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 BMX gene expression was significantly between normal breast tissues 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 metastatic tumor tissues of patients with metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

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

Identification of differentially expressed genes across datasets in cancer can be difficult and more challenging than when doing so in benign organ tissue and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked how gene expression was most significantly different between brain metastases from patients with breast cancer and normal breast tissue as opposed to primary tumors of the breast, by comparing global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁴. Using this strategy, we identified the tyrosine kinase BMX 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 in expression between brain

metastases and normal breast tissues, BMX ranked 79 out of 41093 total transcripts (Table 1), equating to 99.8% differential expression. Differential expression of BMX in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant ($p=2.33E-12$).

Next, we queried a second microarray dataset⁵ to determine whether we could validate differential expression of BMX in breast metastatic tissues of humans with breast cancer. When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, BMX ranked 519 out of 22575 total transcripts (Table 2), equating to 97.7% differential expression. Differential expression of BMX in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 2; $p=2.72E-03$). Thus, differential expression of BMX was conserved both when compared to normal tissues of the breast and to primary tumors of the breast in patients with metastatic breast cancer, in two independent microarray datasets^{4,5}.

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

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

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the tyrosine kinase BMX as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer both when compared to the normal breast and to primary tumors of the breast; BMX expression, as compared to the benign breast, was significantly lower in metastasis to the brain.

Discussion

We provided evidence here that the tyrosine kinase BMX⁷⁻¹⁰, encoded by BMX, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. Evaluation of the effects of ectopic expression of BMX in mouse models of metastatic breast cancer on metastasis to the central nervous system are merited.

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. 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.
5. Harrell, J.C., Prat, A., Parker, J.S., Fan, C., He, X., Carey, L., Anders, C., Ewend, M. and Perou, C.M., 2012. Genomic analysis identifies unique signatures predictive of brain, lung, and liver relapse. *Breast cancer research and treatment*, 132(2), pp.523-535.
6. Awada, A., Colomer, R., Inoue, K., Bondarenko, I., Badwe, R.A., Demetriou, G., Lee, S.C., Mehta, A.O., Kim, S.B., Bachelot, T. and Goswami, C., 2016. Neratinib plus paclitaxel vs trastuzumab plus paclitaxel in previously untreated metastatic ERBB2-positive breast cancer: the NEfERT-T randomized clinical trial. *JAMA oncology*, 2(12), pp.1557-1564.
7. Tamagnone, L., Lahtinen, I., Mustonen, T., Virtaneva, K., Francis, F., Muscatelli, F., Alitalo, R., Smith, C.I., Larsson, C. and Alitalo, K., 1994. BMX, a novel nonreceptor tyrosine kinase gene of the BTK/ITK/TEC/TXK family located in chromosome Xp22. 2. *Oncogene*, 9(12), pp.3683-3688.
8. Smith, C.E., Islam, T.C., Mattsson, P.T., Mohamed, A.J., Nore, B.F. and Vihinen, M., 2001. The Tec family of cytoplasmic tyrosine kinases: mammalian Btk, Bmx, Itk, Tec, Txk and homologs in other species. *Bioessays*, 23(5), pp.436-446.
9. Qiu, Y., Robinson, D., Pretlow, T.G. and Kung, H.J., 1998. Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells. *Proceedings of the National Academy of Sciences*, 95(7), pp.3644-3649.
10. Saharinen, P., Ekman, N., Sarvas, K., Parker, P., Alitalo, K. and Silvennoinen, O., 1997. The Bmx tyrosine kinase induces activation of the Stat signaling pathway, which is specifically inhibited by protein kinase Cδ. *Blood, The Journal of the American Society of Hematology*, 90(11), pp.4341-4353.

Rank	ID	p-value	t	B	Gene	Gene name
79	A_23_P253602	2.33E-12	-9.3964367	18.009456	BMX	BMX non-receptor tyrosine kinase

Table 1: BMX is differentially expressed in brain metastases in patients with 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.

Rank	ID	p-value	t	B	Gene	Gene name
519	525	2.72E-03	-3.65	-1.47489	BMX	BMX non-receptor tyrosine kinase

Table 2: BMX is differentially expressed in brain metastases of patients with 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, gene and gene name are listed in this chart.

Figure 1: BMX is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to normal breast tissues.

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