Klotho is differentially expressed in primary tumors and brain metastases in humans with 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 report here the differential expression of Klotho, encoded by KL, in the primary tumors and brain metastases in humans with breast cancer. KL mRNA was present at significantly reduced quantities in both primary tumors and brain metastatic tissues as compared to normal breast tissues. These data combined suggest that down-regulation of Klotho is a conserved event during transformation of breast tissues and progression to central nervous system metastasis and further point to potential importance of Klotho repression during progression of human breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, KL, Klotho, 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 trial 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 primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and decreased expression of Klotho, encoded by KL, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE109169⁵ 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. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast and; analysis was performed using platform GPL5175. The tissues from this dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). 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 KL 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 tissues from patients with metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of primary and metastatic tumors in human metastatic breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

KL 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 Klotho, KL, 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, KL ranked 30 out of 41093 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of KL in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant ($p=2.63E-16$).

KL is differentially expressed in the primary tumors of patients with breast cancer.

Next, we queried a second microarray dataset⁵ generated using primary tumors and normal breast tissues from patients with breast cancer to determine whether differential expression of Klotho was specific to metastatic tissues or could be found in primary tumors as well. We observed significant differential expression of KL in primary tumors of patients with breast cancer. When sorting each of the genes expressed in primary tumors of the breast based on significance of difference as compared to normal breast tissues in patients with breast cancer, KL ranked 82 out of 19076 total transcripts (Table 2), equating to 99.6% differential expression. Differential expression of KL in the primary tumors of patients with breast cancer was statistically significant (Table 2; $p=4.88E-15$). Thus, differential expression of KL was conserved in the brain metastases and primary tumors of patients breast cancer.

KL is expressed at significantly lower levels in the brain metastases and primary tumors in humans with breast cancer.

We obtained exact mRNA expression levels for KL, in normal breast tissues and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in KL expression in brain metastatic tissues. KL 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; $p<0.0001$).

We also obtained exact mRNA expression levels for KL, in normal breast tissues and in primary tumors of patients with breast cancer to determine direction and statistical significance of change in KL expression in primary tumors of the breast. KL was expressed at lower levels in the primary tumors of patients with breast cancer as compared to normal breast tissues, and this difference was statistically significant (Figure 2; $p<0.0001$).

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

Discussion

We provided evidence here that Klotho⁷, encoded by KL, is among the genes whose expression is most different in the brain metastases and primary tumors of patients with brain metastatic brain cancer. Evaluation of the effects of ectopic expression of KL in mouse models of metastatic breast cancer on metastasis to the central nervous system are merited. Differential expression and down-regulation of Klotho in primary tumors of the breast and metastasis to the brain suggests that repression of Klotho may be important for the progression of human breast cancer, but this remains to be experimentally evaluated.

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. 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.
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. Kurosu, H., Yamamoto, M., Clark, J.D., Pastor, J.V., Nandi, A., Gurnani, P., McGuinness, O.P., Chikuda, H., Yamaguchi, M., Kawaguchi, H. and Shimomura, I., 2005. Suppression of aging in mice by the hormone Klotho. *Science*, 309(5742), pp.1829-1833.

Rank	ID	p-value	t	B	Gene	Gene name
30	A_23_P503064	2.63E-16	-12.3131601	26.774774	KL	klotho

Table 1: KL 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	FC	Gene	Gene name
82	3484895	4.88E-15	-1.1E+01	24.036654	0.84 ± 0.05	KL	Klotho

Table 2: KL is differentially expressed in primary tumors of the breast when compared to normal breast tissue.

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: KL 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 KL in normal breast tissues (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean KL 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: KL is expressed at significantly lower levels in the primary tumors of patients with breast cancer as compared to normal breast tissues.

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