Aldolase B is differentially expressed in brain metastatic breast cancer.

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Brain metastases affect up to 34% of breast cancer patients treated with trastuzumab¹. Limited treatment options are available for clinical control of brain metastatic breast cancer²⁻⁴. We mined published microarray data^{5,6} to identify genes associated with metastasis to the brain in human breast cancer. This unbiased, global gene expression analysis identified differential expression of aldolase B, encoded by the gene Aldob, an enzyme functioning in the metabolism of fructose^{7,8}, as a transcriptional feature of brain metastasis in patients with breast cancer. Aldolase B may be of relevance to processes underlying metastasis to the brain in human metastatic breast cancer.

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

Brain metastases are a clinical problem for patients with breast cancer²⁻⁴. One study reported a central nervous system metastatic frequency of 34% for patients with breast cancer who had been treated with trastuzumab¹. Unbiased approaches to describe the transcriptional landscape of brain metastases in patients with breast cancer are required. We mined published microarray data^{5,6} to perform blind, global comparative transcriptome analysis of primary tumors and brain metastases in patients with human breast cancer. We identified aldolase B, an enzyme functioning in the metabolism of fructose, as among the genes whose expression was most significantly different in the brain metastases of patients with breast cancer as compared to primary tumors of the breast.

Methods

We used datasets GSE125989⁵ and GSE26338⁶ 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. 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 Aldob 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 harnessed the power of blind, systems-level comparative transcriptome analysis to discover genes associated with metastasis to the brain in human breast cancer using published microarray data^{5,6}.

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

We identified the gene encoding aldolase B, Aldob, as among the genes whose expression was most quantitatively different in brain metastases when compared to primary tumors of the breast in patients with breast cancer⁵. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, Aldob ranked 487 out of 22277 total transcripts (Table 1), equating to 97.8% differential expression. Differential expression of Aldob in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=7.6E-03$).

We queried a second microarray dataset⁶ to attempt to validate transcriptome-wide differential expression of Aldob in brain metastatic breast cancer. We again found that Aldob was among the genes whose expression was most significantly different when comparing primary tumors of the breast to brain metastases from patients with metastatic breast cancer. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, Aldob ranked 1320 out of 22575 total transcripts (Table 1), equating to 94.2% differential expression.

Differential expression of Aldob in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=1.14E-02$).

Aldob is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

We obtained exact mRNA expression levels for Aldob in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in Aldob gene expression in brain metastatic tissues. Aldob was expressed at higher levels in brain metastases from patients with breast cancer as compared to primary tumors of the breast (Figure 1). Increased expression of Aldob in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1; $p=0.0023$). We calculated a mean fold change of 1.64 ± 0.65 in Aldob expression in brain metastases when compared to primary tumors of the breast (Table 1).

Thus, through comparative transcriptome analysis of tumor and metastatic tumor tissues of patients with breast cancer using published microarray data^{5,6}, we found here that Aldob was among the genes whose expression was most significantly different when comparing brain metastases to primary tumors of the breast in patients with breast cancer.

Discussion

Aldolase B functions in the metabolism of fructose by catalyzing the cleavage of both fructose-1-bisphosphate and fructose-1,6-bisphosphate; cleavage of fructose-1-phosphate produces D-glyceraldehyde and dihydroxyacetone phosphate^{7,8}. Glyceraldehyde can be utilized in the Krebs' cycle after conversion to glyceraldehyde-3-phosphate, or it can be converted to fructose-1,6-bisphosphate; fructose-1,6-bisphosphate can function as a glycolytic intermediate metabolite or it can be utilized for gluconeogenesis⁸. The aldolase B gene is mutated in hereditary fructose intolerance (HFI), a genetically transmitted disorder resulting in gastrointestinal manifestations of fructose intolerance, including nausea, vomiting and severe abdominal pain in conjunction with hypoglycemia⁹. Depletion of all three aldolases, aldolase A, aldolase B and aldolase C, results in activation of the AMP-activated protein kinase AMPK, indicating aldolases can regulate AMPK function and thus sensing of glucose availability¹⁰. In human cancer, aldolase B can function support development of metastasis to the liver in cancer of the colon^{11,12}; aldolase B expression is significantly higher in liver metastases as compared to normal tissue of the colon or primary tumors of the colon; expression of aldolase B could be controlled by GATA6 and by changes in fructose concentration^{11,12}. Importantly, depletion of aldolase B in the HCT116, CRC119, and CRC57 colorectal cancer cell lines resulted in significant reduction in tumor growth in the liver when these cell lines were injected intrahepatically in mice¹². Thus, aldolase B functions in the metabolism of the fructose sugar and expression of aldolase B is up-regulated in liver metastases in human colon cancer where it promotes fructose metabolism and in mouse models of colorectal cancer, tumor growth in the liver.

By mining published microarray data^{5,6} generated using primary and metastatic tumor tissues of patients with breast cancer, we found that aldolase B was among the genes whose expression was most significantly different in metastases to the brain as compared to primary tumors of the breast; aldolase B expressed at significantly higher levels in brain metastatic tissues. It would be of interest to evaluate metabolism of fructose in brain metastases from patients with metastatic breast cancer, and determine whether genetic depletion of aldolase B in murine models of metastatic breast cancer reduce metastasis to the brain.

References

1. 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.
2. 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.
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. 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.
5. 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.
6. 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.
7. Tolan, D.R. and Penhoet, E.E., 1986. Characterization of the human aldolase B gene. *Molecular biology & medicine*, 3(3), pp.245-264.
8. Cox, T.M., 1994. Aldolase B and fructose intolerance. *The FASEB journal*, 8(1), pp.62-71.
9. Cornblath, M., Rosenthal, I.M., Reisner, S.H., Wybregt, S.H. and Crane, R.K., 1963. Hereditary fructose intolerance. *New England Journal of Medicine*, 269(24), pp.1271-1278.
10. Zhang, C.S., Hawley, S.A., Zong, Y., Li, M., Wang, Z., Gray, A., Ma, T., Cui, J., Feng, J.W., Zhu, M. and Wu, Y.Q., 2017. Fructose-1, 6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature*, 548(7665), pp.112-116.
11. Leong, I., 2018. ALDOB promotes liver metastases development. *Nature Reviews Endocrinology*, 14(7), pp.380-380.
12. Bu, P., Chen, K.Y., Xiang, K., Johnson, C., Crown, S.B., Rakhilin, N., Ai, Y., Wang, L., Xi, R., Astapova, I. and Han, Y., 2018. Aldolase B-mediated fructose metabolism drives metabolic reprogramming of colon cancer liver metastasis. *Cell metabolism*, 27(6), pp.1249-1262

Rank	ID	p-value	t	B	FC	Gene	Gene name
487	204704_s_at	7.6E-03	2.8355644	-2.4971	1.64 ± 0.65	ALDOB	aldolase, fructose-bisphosphate B

Table 1: Brain metastases from patients with breast cancer differentially express Aldob.

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 Aldob 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
1320	8024	1.14E-02	2.89	-2.82416	ALDOB	aldolase B, fructose-bisphosphate

Table 2: Brain metastases from patients with breast cancer differentially express Aldob.

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: Aldolase B is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast in patients with breast cancer.

The mRNA expression level of aldolase B (Aldob) 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 Aldob 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.