The vitamin D receptor 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 the vitamin D (1α , $25(\text{OH})_2\text{D}_3$) receptor, encoded by VDR, as a transcriptional feature of brain metastasis in patients with breast cancer. Primary tumor expression of VDR was significantly correlated with overall survival in patients with breast cancer. VDR 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, VDR, 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 the vitamin D receptor 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 VDR 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. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of VDR mRNA expression levels with overall survival in $n=1402$ breast patients. 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}.

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

We identified the gene encoding the vitamin D ($1\alpha,25(\text{OH})_2\text{D}_3$) receptor, VDR, 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, VDR ranked 174 out of 22277 total transcripts (Table 1), equating to 99.2% differential expression. Differential expression of VDR in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=1.16\text{E-}03$).

We queried a second microarray dataset⁶ to attempt to validate transcriptome-wide differential expression of VDR in brain metastatic breast cancer. We again found that VDR 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, VDR ranked 1663 out of 22575 total transcripts (Table 1), equating to 92.6% differential expression.

Differential expression of VDR in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=1.65E-02$).

VDR 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.

We obtained exact mRNA expression levels for VDR in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in VDR gene expression in brain metastatic tissues. VDR was expressed at lower levels in brain metastases from patients with breast cancer as compared to primary tumors of the breast (Figure 1). Decreased expression of VDR in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1; $p=0.0018$). We calculated a mean fold change of 0.79 ± 0.20 in VDR expression in brain metastases when compared to primary tumors of the breast (Table 1).

VDR expression associates with survival outcomes in breast cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between VDR mRNA expression levels with survival outcomes in patients with breast cancer⁷. In 1402 breast cancer patients, we found a statistically significant correlation between VDR expression and overall survival (OS) (Figure 2; log rank p -value: 0.0049 for overall survival, hazard ratio: 0.74 (0.59 - 0.91) (Fig. 2)). Lower VDR mRNA levels in primary tumors of the breast, like which we observed in metastases to the brain, were a negative prognostic indicator in patients with breast cancer with respect to overall survival. Median OS was 130 months for patients with low tumor expression of VDR while median OS was 80 months for patients with high tumor expression of VDR (Table 3).

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 VDR 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, that VDR was expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast, and that VDR expression significantly correlated with overall survival in patients with breast cancer .

Discussion

Existing published data regarding the role of vitamin D and the vitamin D receptor in human breast cancer is conflicting⁸.

We provided evidence here that the vitamin D receptor was among the genes whose expression was most significantly different in metastases to the brain as compared to primary tumors of the breast; VDR was expressed at significantly lower levels in brain metastatic tissues, and VDR expression was significantly correlated with overall survival, with significantly inferior overall survival in patients whose primary tumors expressed low levels of VDR. It would be of interest to evaluate $1\alpha,25(\text{OH})_2\text{D}_3$ metabolism in brain metastases from patients with metastatic breast cancer, and determine whether ectopic expression of VDR in murine models of metastatic breast cancer reduces 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. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*, 123(3), pp.725-731.
8. Narvaez, C.J., Matthews, D., LaPorta, E., Simmons, K.M., Beaudin, S. and Welsh, J., 2014. The impact of vitamin D in breast cancer: genomics, pathways, metabolism. *Frontiers in physiology*, 5, p. 213.

Rank	ID	p-value	t	B	FC	Gene	Gene name
174	213692_s_at	1.16E-03	-3.5416774	-0.867	0.79 ± 0.20	VDR	vitamin D (1,25-dihydroxyvitamin D3) receptor

Table 1: Brain metastases from patients with metastatic breast cancer differentially express VDR 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, fold change (FC) of VDR 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
1663	6317	1.65E-02	-2.71	-3.16314	VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor

Table 2: Brain metastases from patients with metastatic breast cancer differentially express VDR 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: VDR is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with breast cancer.

The mRNA expression level of the vitamin D receptor (VDR) 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 VDR 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: VDR expression correlates with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=1402$ total patients stratified into two groups, based on low or high expression of VDR in patient primary tumors. The log rank p -value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on VDR expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
80	130

Table 3: Median overall survival is superior in breast cancer patients with high primary tumor expression of VDR.

Overall survival of $n=1402$ breast cancer patients based on stratification into low or high tumor expression of VDR is listed in this chart.