The progesterone receptor is differentially expressed in brain 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 the progesterone receptor, encoded by PGR, was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. We observed a significant correlation between PGR expression in primary tumors of the breast and distant metastasis-free survival, as well as overall survival. Down-regulation of PGR may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer, and irrespective of its importance, appears to be a transcriptional event generally associated with metastasis to the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, PGR, progesterone receptor, 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 cancer². 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, 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 the progesterone receptor, encoded by PGR, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE52604⁵ 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 PGR 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). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁶ for correlation of PGR mRNA expression levels with distant metastasis-free survival (DMFS) in $n=1746$ breast cancer patients, and overall survival in $n=1402$ breast patients.

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^{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.

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

We identified the progesterone receptor, PGR, 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 as compared to the primary tumors of patients with brain metastatic breast cancer, PGR ranked 1476 out of 22297 total transcripts (Table 1), equating to 93.4% differential expression. Differential expression of PGR in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=3.97E-02$).

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 PGR 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 metastases⁵ revealed that PGR 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 breast tissues, PGR ranked 249 out of 41093 total transcripts (Table 2), equating to 99.998% differential expression. Differential expression of PGR in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=5.42E-10$).

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

We obtained exact mRNA expression levels for PGR, 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 PGR expression in brain metastatic tissues. PGR 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; $p<0.0001$).

PGR expression associates with survival outcomes in breast cancer.

We performed Kaplan-Meier survival analysis⁶ to evaluate correlation between PGR mRNA expression levels with survival outcomes in patients with breast cancer. In 1746 breast cancer patients, we found a statistically significant correlation between PGR expression and distant metastasis-free survival (DMFS) (Figure 2; log rank p -value: $1.4e-10$ for distant metastasis-free survival, hazard ratio: 0.53 (0.44 - 0.65) (Fig. 2)). Lower PGR 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. Median DMFS was 58.88 months for patients with low tumor expression of PGR while median DMFS was 144.59 months for patients with high tumor expression of PGR (Table 3).

We also performed Kaplan-Meier survival analysis to evaluate correlation between PGR mRNA expression levels with overall survival outcomes in patients with breast cancer⁶. In 1402 breast cancer patients, we found a statistically significant correlation between PGR expression and overall survival (OS) (Figure 3; log rank p -value: $1.1e-05$ for overall survival, hazard ratio: 0.62 (0.5 - 0.77) (Fig. 3)). Lower PGR 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 70.68 months for patients with low tumor expression of PGR while median OS was 138 months for patients with high tumor expression of PGR (Table 4).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified PGR 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; PGR was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast. PGR expression in primary tumors of the breast in a large cohort of patients demonstrated a significant correlation with both distant metastasis-free survival and overall survival, with higher expression of PGR correlating with improved survival in humans with breast cancer.

Discussion

One study described a negative relationship between membrane progesterone receptor alpha expression and TNM stage in triple negative breast cancer, as well as an inhibitory effect of progesterone on tumor growth and metastasis of triple negative breast cancer cells in xenografted nude mice⁷.

We provided evidence here that the progesterone receptor, encoded by PGR, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer and that low expression of PGR, like that which we observed in brain metastatic tissues, is significantly correlated with worse distant-metastasis free survival and overall survival. Independent evaluation of the effects of ectopic expression of PGR in mouse models of metastatic breast cancer on metastasis to the central nervous system are merited.

References

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Rank	ID	p-value	t	B	Gene	Gene name
1476	208305_at	3.97E-02	-2.1382111	-3.8857	PGR	progesterone receptor

Table 1: PGR is differentially expressed in brain metastases in 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.

Rank	ID	p-value	t	B	Gene	Gene name
249	A_32_P49199	5.42E-10	-7.7835477	12.708821	PGR	progesterone receptor

Table 2: PGR is differentially expressed in brain metastases of patients with metastatic breast cancer 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: PGR is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of PGR 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 PGR 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: PGR expression correlates with distant metastasis-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival (DMFS) for $n=1746$ total patients stratified into two groups, based on low or high expression of PGR in patient primary tumors. The log rank p -value denoting statistical significance of difference in distant metastasis-free 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 PGR 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)
58.88	144.59

Table 3: Median distant metastasis-free survival is superior in breast cancer patients with high primary tumor expression of PGR.

The distant metastasis-free survival of $n=1746$ breast cancer patients based on stratification into low or high tumor expression of PGR is listed in this chart.

Figure 3: PGR 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 PGR 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 PGR 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)
70.68	138

Table 4: Median overall survival is superior in breast cancer patients with high primary tumor expression of PGR.

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