OR2T12 is a differentially expressed gene in brain metastatic human 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 for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that olfactory receptor 2 subfamily T member 12, encoded by OR2T12, 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. OR2T12 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Modulation of OR2T12 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, olfactory receptor 2 subfamily T member 12, OR2T12, 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 trastuzumab⁶. 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 differential and increased expression of the gene encoding olfactory receptor 2 subfamily T member 12, OR2T12, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE22384⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE10893 was generated using Agilent-011521 Human 1A Microarray G4110A technology with $n=11$ primary breast tumors and $n=3$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. GSE22384 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=9$ normal breast tissues adjacent to tumor and $n=12$ tumors of the breast from patients with breast cancer; analysis was performed using platform GPL8264. 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 OR2T12 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test.

Results

We performed global comparative transcriptome analysis of primary and metastatic tumor tissues of patients with breast cancer and 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.

OR2T12 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified olfactory receptor 2 subfamily T member 12, encoded by OR2T12, as a differentially expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). 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, OR2T12 ranked 85 out of 17418 total

transcripts (Chart 1), equating to 99.5% differential expression. Differential expression of OR2T12 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.72E-04$).

To attempt to validate differential transcriptome-wide differential expression of OR2T12 in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues and primary tumors of the breast. Again, we identified OR2T12 as a differentially expressed gene in the tumor tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in primary tumors of the breast based on significance of difference as compared to normal breast tissues, OR2T12 ranked 54468 out of 243504 total transcripts (Chart 2), equating to 77.6% differential expression. Differential expression of OR2T12 in the primary tumors of patients with breast cancer was statistically significant (Chart 2; $p=3.06e-2$). Thus, differential expression of OR2T12, transcriptome-wide, in the primary tumors of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

OR2T12 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for OR2T12, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in OR2T12 expression in brain metastatic tissues. OR2T12 was expressed at higher 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; $p=0.000068$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified olfactory receptor 2 subfamily T member 12, encoded by OR2T12, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases and primary tumors of patients with breast cancer; we observed significantly increased expression of OR2T12 in brain metastases as compared to primary tumors of the breast.

Discussion

We provided evidence here that carbonic anhydrase 8, encoded by OR2T12, is among the genes whose expression is most different in the brain metastases and primary tumors of humans with breast cancer, that OR2T12 mRNA is present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Evaluation of the effects of genetic depletion of OR2T12 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of OR2T12 expression may be relevant to the processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.

References

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Rank: 85
Probe ID: 10384
p-value: 1.72E-04
t: -4.88
B: 1.026712
Gene: OR2T12
Gene name: olfactory receptor 2 subfamily T member 12

Chart 1: OR2T12 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, the probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 54468
probe ID: 158219
p-value: 3.06e-2
t: 2.31
B: -4.0079573
Gene: OR2T12
Gene name: olfactory receptor 2 subfamily T member 12

Chart 2: OR2T12 is differentially expressed in human breast cancer when comparing primary tumors of the breast to normal breast tissues.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: OR2T12 is expressed at significantly higher levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of OR2T12 in primary tumors of the breast (left) and in brain metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in OR2T12 expression between primary tumors of the breast and brain metastases is listed above as a *p*-value.