RAB28 is a differentially expressed gene in human 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 for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that ras-related protein Rab 28, encoded by RAB28, was among the genes whose expression was most quantitatively different in the brain metastases of patients with metastatic breast cancer. RAB28 mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of RAB28 in primary tumors was significantly correlated with patient overall survival in patients with breast cancer. Modulation of RAB28 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, ras-related protein Rab 28, RAB28, 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 decreased expression of the gene encoding ras-related protein Rab 28, RAB28, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GS52604⁵ 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 $n=4$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer; analysis was 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 RAB28 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. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of RAB28 mRNA expression levels in primary tumors of the breast with overall survival in $n=1402$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary tumor tissues of patients with metastatic breast, as well as normal tissues of the breast 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.

RAB28 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 ras-related protein Rab 28, encoded by RAB28, as a differentially expressed gene in the brain 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, RAB28 ranked 123 out of 17418 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of RAB28 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.45e-04$).

Differential gene expression analyses that utilize primary and metastatic tumor tissues to discover genes associated with metastasis can be challenging due to the relatively heterogeneous cellular composition and constitution of the tumor as compared to normal, untransformed (benign) tissues. Thus, to attempt to validate differential transcriptome-wide differential expression of RAB28 in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues rather than primary tumors of the breast to brain metastases. Again, we identified RAB28 as a differentially expressed gene in the brain metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to normal breast tissues, RAB28 ranked 11116 out of 41093 total transcripts (Chart 2), equating to 72.9% differential expression. Differential expression of RAB28 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=7.96e-03$). Thus, differential expression of RAB28, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, both when compared to benign and transformed breast tissues. This suggests that RAB28 differential expression in human brain metastatic breast cancer is a *bona fide* biological process.

RAB28 is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for RAB28, 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 RAB28 expression in brain metastatic tissues. We observed significantly decreased expression of RAB28 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: RAB28 was expressed at -0.54 ± 0.36 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -1.50 ± 0.20 AU in brain metastatic tissues (Figure 1). The difference in RAB28 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000269$).

RAB28 expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=1402$ breast cancer patients in total, to evaluate whether RAB28 tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of RAB28 and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of RAB28 possessed median OS of 69.99 months, while patients whose tumors expressed high levels of RAB28 possessed median OS of 110.4 months. This difference in OS based on RAB28 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value 0.04; hazard ratio: 0.78 (0.62-0.99)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified ras-related protein Rab 28, encoded by RAB28, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer, when compared to primary tumors of the breast and to normal breast tissues, respectively; we observed significantly decreased expression of RAB28 in brain metastases as compared to primary tumors of the breast. Further, we found a correlation between RAB28 expression and patient survival outcomes, as overall survival was greater in patients whose primary tumors expressed higher levels of RAB28 as compared to patients whose primary tumors expressed lower levels of RAB28.

Discussion

We provided evidence here that RAB28 is among the genes whose expression is most different in the brain metastases of patients with metastatic breast cancer, that RAB28 mRNA is present at significantly decreased quantity in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor RAB28 expression is correlated with patient survival outcomes in human breast cancer. Rab28 is a small GTPase with at least 3 mRNA variants each of which differ at their carboxy terminus, and is suggested to be farnesylated^{8,9}. Evaluation of the effects of genetic depletion of RAB28 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of RAB28 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.

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Rank: 123
Probe ID: 11928
p-value: 2.45e-04
t: 4.71
B: 0.703801
Gene: RAB28
Gene name: ras-related protein Rab 28

Chart 1: RAB28 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: 11116
probe ID: A_32_P7204
p-value: 7.96e-03
t: -2.7723023
B: -3.146313
Gene: RAB28
Gene name: ras-related protein Rab 28

Chart 2: RAB28 is differentially expressed in brain metastatic breast cancer when comparing brain metastases 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: RAB28 is expressed at significantly lower levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of RAB28 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 RAB28 expression between primary tumors and brain metastases is $p=0.000269$.

Figure 2: RAB28 primary tumor expression is correlated with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total breast cancer patients stratified into two groups, based on low or high expression of RAB28 in patient primary tumor. 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 RAB28 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.

1 Low RAB28 expression: 69.99 months

2 High RAB28 expression: 110.4 months

3
4 **Chart 3: Median overall survival is inferior in patients with low primary tumor expression of RAB28.**

5 The median OS (overall survival) of patients with low primary tumor expression of RAB28 and high
6 primary tumor expression of RAB28 is listed in this chart, for $n=1402$ breast cancer patients.