ARSK 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 arylsulfatase family member K, encoded by ARSK, 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. ARSK mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of ARSK in primary tumors was significantly correlated with patient overall survival. Modulation of ARSK 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, arylsulfatase family member K, ARSK, 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 arylsulfatase family member K, ARSK, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE43837⁵ 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. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary tumors of the breast and $n=19$ 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 ARSK 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 ARSK mRNA expression levels with overall survival in $n=626$ patients; auto cut-off was selected.

Results

We performed global comparative transcriptome analysis of metastatic tumor tissues of patients with 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.

ARSK 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 arylsulfatase family member K, encoded by ARSK, 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, ARSK ranked 114 out of 17418 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of ARSK in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.28\text{e-}04$).

To attempt to validate differential transcriptome-wide differential expression of ARSK in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵; again, we identified ARSK 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 primary tumors of the breast, ARSK ranked 293 out of 61359 total transcripts (Chart 2), equating to 99.5% differential expression. Differential expression of ARSK in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=0.00143916$). Thus, transcriptome-wide differential expression of ARSK in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, indicating that this may likely be a *bona fide* biological phenomena associated with metastatic breast cancer in humans.

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

We obtained exact mRNA expression levels for ARSK, 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 ARSK expression in brain metastatic tissues. We observed significantly lower expression of ARSK in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: ARSK was expressed at -0.34 ± 0.47 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -1.53 ± 0.27 AU in brain metastatic tissues. The difference in ARSK mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000406$).

ARSK expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in 626 breast cancer patients in total, to evaluate whether ARSK tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of ARSK and overall survival (OS) in patients with breast cancer, in the upper survival quartile (Figure 2). Patients whose primary tumors expressed low levels of ARSK possessed median OS of 90 months, while patients whose tumors expressed high levels of ARSK possessed median OS of 62.07 months. This difference in OS based on ARSK tumor expression in patients with breast cancer approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.059; hazard ratio: 1.39 (0.99-1.97)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified arylsulfatase family member K, encoded by ARSK, 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; we observed significantly decreased expression of ARSK in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between ARSK expression and patient survival outcomes, as overall survival was significantly greater in patients whose tumors expressed lower levels of ARSK as compared to patients whose tumors expressed higher levels of ARSK.

Discussion

We provided evidence here that arylsulfatase family member K, encoded by ARSK, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that ARSK mRNA is present at significantly decreased quantities in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor ARSK expression is significantly correlated with patient survival outcomes in human breast cancer. ARSK is a lysosomal arylsulfatase, a family of enzymes that catalyzes the hydrolysis of sulfate esters on substrates including

glycosaminoglycans such as heparan sulfate, which all contain a formylglycine residue at their catalytic site, contained with a C-T/S/C/A-P-S-R motif known as a sulfatase signature⁸. Evaluation of the effects of genetic depletion of ARSK in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of ARSK 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: 114
Probe ID: 20492
p-value: 2.28e-04
t: 4.74
B: 0.768707
Gene: ARSK
Gene name: arylsulfatase family member K

Chart 1: ARSK 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: 293
probe ID: 1569133_3p_x_at
p-value: 0.00143916
t: 3.4069573
B: -1.12634
Gene: ARSK
Gene name: arylsulfatase family member K

Chart 2: ARSK is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

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: ARSK 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 ARSK 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 ARSK expression between primary tumors of the breast and brain metastases is $p=0.000406$.

Figure 2: Significant correlation between ARSK primary tumor expression and overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=626$ total patients with breast cancer stratified into two groups, based on low or high expression of ARSK in patient primary tumors, in the upper survival quartile. 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 ARSK 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 ARSK expression: 90 months
High ARSK expression: 62.07 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of ARSK.

The median OS (overall survival) of patients with low primary tumor expression of ARSK and high primary tumor expression of ARSK is listed in this chart, for $n=626$ breast cancer patients, in the upper survival quartile.