SEC61A2 is differentially expressed in the brain metastases of patients with 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 Sec61 translocon alpha 2 subunit encoded by SEC61A2 was among the genes whose expression was most different in the brain metastatic tumor tissues of patients with metastatic breast cancer. SEC61A2 messenger RNA was present at significantly increased quantities in metastasis to the brain when compared to normal breast tissues. SEC61A2 was also differentially expressed in primary tumors of the breast when compared to normal breast tissues. Up-regulation of SEC61A2 may be important for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer, and these data suggest that a specific function of the Sec61 translocon may be important for central nervous system metastasis, or that the biosynthesis and transport of integral membrane and secreted proteins more broadly is in higher demand during this process.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, SEC61A2, 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 metastatic breast cancer^{4,5}. We discovered transcriptome-wide differential expression of SEC61A2 in the brain metastases of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE22384⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. 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 was performed using platform GPL6480. GSE22384 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=9$ normal breast tissues and $n=12$ primary tumors of the breast; 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 SEC61A2 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).

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 metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

SEC61A2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the Sec61 translocon alpha 2 subunit encoded by SEC61A2 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, SEC61A2 ranked 32 out of 22277 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of SEC61A2 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=3.76E-16$).

We asked whether differential expression of SEC61A2 was specific to brain metastasis or whether SEC61A2 differential expression could also be found in primary tumors of the breast. When sorting each of the genes expressed in primary tumors of the breast based on significance of difference in expression as compared to primary tumors of the breast, SEC61A2 ranked 7117 out of 243504 total transcripts (Table 2), equating to 97.1% differential expression. Differential expression of SEC61A2 in the primary tumors of patients with breast cancer was statistically significant (Table 2; $p=1.64E-04$).

SEC61A2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for SEC61A2, 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 SEC61A2 expression in brain metastatic tissues relative to the untransformed breast. SEC61A2 mRNA was present 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; $p < 0.0001$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified SEC61A2 as a differentially expressed gene in the brain metastases of patients with metastatic breast cancer when compared to tissues of the breast, transformed and benign. SEC61A2 was expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast.

Discussion

We provided evidence here that the Sec61 translocon alpha 2 subunit encoded by SEC61A2 is among the genes whose expression is most different in the brain metastases of patients with metastatic breast cancer. The Sec61 translocon is a transmembrane channel-type protein of the endoplasmic reticulum that functions in highly orchestrated process by which proteins synthesized by the ribosomes and destined for insertion into the plasma membrane - integral membrane proteins - or destined for extracellular secretion - secreted proteins - are transported across the lipid bilayer of the endoplasmic reticulum following or in concert with translation⁷⁻¹⁰. One interesting function that has been described for the Sec61 translocon includes transport of the epidermal growth factor receptor to the nucleus¹¹. SEC61A2 may be of relevance to any number of steps required for metastasis to the brain, and up-regulation of SEC61A2 in metastatic tissues of the brain in conjunction with differential expression of SEC61A2 in primary tumors of the breast relative to normal breast tissue suggests that changes in SEC61A2 expression at some point during progression of primary tumor to brain metastases - including exit from the breast, entry into the vasculature or lymphatics, or breach of the blood-brain barrier - may be important for colonization of the brain with primary tumor-derived clones.

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Rank	ID	p-value	t	B	Gene	Gene name
32	A_23_P161197	3.76E-16	12.1916877	26.433234	SEC61A2	Sec61 translocon alpha 2 subunit

Table 1: SEC61A2 is differentially expressed in brain metastases in metastatic breast cancer when compared to normal breast tissues.

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 SEC61A2 expression between the brain metastases of patients with breast cancer and normal breast tissues, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
7117	68716	1.64E-04	4.54	0.8828619	SEC61A2	Sec61 alpha 2 subunit (S. cerevisiae)

Table 2: SEC61A2 is differentially expressed in primary tumors of the breast in patients with 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: SEC61A2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of SEC61A2 in normal breast tissue (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean SEC61A2 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal (benign) tissues of the breast, a *p*-value, listed above.