Differential expression of integral membrane protein 2A in cancers of the breast.

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Breast cancer affects women at relatively high frequency¹. We mined published microarray datasets^{2,3} to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We report here significant differential expression of the gene encoding integral membrane protein 2A, ITM2A, when comparing primary tumors of the breast to the tissue of origin, the normal breast. ITM2A was also differentially expressed in the tumor cells of patients with triple negative breast cancer. ITM2A mRNA was present at significantly lower quantities in tumors of the breast as compared to normal breast tissue. Analysis of human survival data revealed that expression of ITM2A in primary tumors of the breast was correlated with overall survival in patients with basal subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by molecular subtype. ITM2A may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, ITM2A, integral membrane protein 2A, systems biology of breast cancer, targeted therapeutics in breast cancer.

Invasive breast cancer is diagnosed in over a quarter of a million women in the United States each year¹ and in 2018, breast cancer was the leading cause of cancer death in women worldwide⁴. While patients with localized breast cancer are provided a 99% 5-year survival rate, patients with regional breast cancer, cancer that has spread to lymph nodes or nearby structures, are provided an 86% 5-year survival rate^{5,6}. Patients with metastasis to distant sites, like the brain, are provided a 27% 5-year survival rate^{5,6}. Understanding how primary tumors are most transcriptionally different from the tissue from which they originate, the breast, can facilitate development of novel diagnostic and therapeutics to promote early detection and enhanced treatment, and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data^{2,3} to understand at the transcriptome level and in an unbiased fashion genes most differentially expressed in primary tumors of the breast as compared to normal breast tissue. Integral membrane protein 2A emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE109169² and GSE38959³ for this differential gene expression analysis of female breast cancer. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast; analysis was performed using platform GPL5175. The tissues from this first dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). GSE38959 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=13$ samples of normal mammary gland ductal cells and $n=30$ samples of tumor cells from patients with triple negative breast cancer; analysis was performed using platform GPL4133. 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 ITM2A expression was significantly different between primary breast tumors and breast tissue using a two-tailed t -test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of ITM2A mRNA expression levels with overall survival (OS) in $n=404$ patients with basal subtype cancer, $n=794$ patients with luminal A subtype cancer, $n=515$ patients with luminal B subtype cancer, and $n=166$ patients with HER2+ cancer.

Results

We performed discovery of genes associated with breast cancer in females by mining two independently published microarray datasets^{2,3}.

ITM2A is differentially expressed in primary tumors of the breast.

Comparison of 25 normal breast tissues to 25 tumors of the breast² from patients with early-onset breast cancer revealed that the gene encoding integral membrane protein 2A, encoded by the ITM2A gene, was among the genes most differentially expressed in tumors of the breast in human breast cancer (Chart 1). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, ITM2A ranked 64 out of 19076 total transcripts, equating to 99.7% differential expression (Chart 1). Differential expression of ITM2A in female breast cancer was statistically significant (Chart 1; $p=1.31\text{E-}15$).

Analysis of a second microarray dataset³, here in tumor cells of patients with triple negative breast cancer as compared to normal mammary gland ductal cells, again revealed significant differential expression of ITM2A in human breast cancer (Chart 2). When sorting each of the genes expressed in the

tumor cells of patients with triple negative breast cancer based on significance of difference as compared to normal mammary gland ductal cells, ITM2A ranked 2217 out of 45015 total transcripts, equating to 95.1% differential expression (Chart 2). Differential expression of ITM2A in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=7.33E-06$). These data suggested that differential expression of ITM2A was not an artifact of a single microarray dataset, nor was it associated with one type of breast cancer, rather a general feature of cancers of the breast.

ITM2A is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for ITM2A from the breast and from breast tumors² to understand the magnitude and direction of ITM2A expression change. ITM2A was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of ITM2A in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). ITM2A was expressed at 8.57 ± 0.61 arbitrary units (AU) in normal breast tissue, while it was expressed at 6.62 ± 0.63 AU in tumors of the breast. We calculated a mean fold change of 0.77 in ITM2A mRNA levels when comparing primary tumors of the breast to normal breast tissues.

ITM2A expression correlates with survival outcomes in basal, luminal A, and luminal B subtype human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate ITM2A mRNA expression levels with survival outcomes in patients with breast cancer, evaluating correlations between molecular subtype and overall survival. We observed a statistically significant correlation between ITM2A expression and overall survival (OS) in patients with basal subtype breast cancer (Figure 2; log rank p -value: 0.0058 for overall survival, hazard ratio: 0.58 (0.4-0.86) (Fig. 2)). ITM2A mRNA levels were a positive prognostic indicator in basal subtype breast cancer patients. Median OS was 39.5 months for basal subtype patients with low tumor expression of ITM2A as compared to basal subtype patients with high tumor expression of ITM2A, who possessed median OS of 97.48 months (Chart 3). We also observed a statistically significant correlation between ITM2A expression and overall survival (OS) in patients with luminal A subtype breast cancer (Figure 2; log rank p -value: 0.02 for overall survival, hazard ratio: 0.69 (0.5-0.95) (Fig. 2)). ITM2A mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. Median OS was 99.6 months for luminal A subtype patients with low tumor expression of ITM2A as compared to luminal A subtype patients with high tumor expression of ITM2A, who possessed median OS of 169.2 months (Chart 3). We also observed a statistically significant correlation between ITM2A expression and overall survival (OS) in patients with luminal B subtype breast cancer (Figure 2; log rank p -value: 0.0046 for overall survival, hazard ratio: 0.6 (0.42-0.86) (Fig. 2)). ITM2A mRNA levels were a negative prognostic indicator in luminal B subtype breast cancer patients, albeit marginally. Median OS was 198.44 months for luminal B subtype patients with low tumor expression of ITM2A as compared to luminal B subtype patients with high tumor expression of ITM2A, who possessed median OS of 197.62 months (Chart 3). ITM2A primary tumor expression was not correlated with overall survival in HER2+ breast cancer (Figure 2; log rank p -value: 0.13 for OS, hazard ratio: 0.64 (0.34-1.14) (Fig. 2)).

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and down-regulation of ITM2A was among the most significant transcriptional features in primary tumors from patients with breast cancer. ITM2A expression in primary tumors of the breast was correlated with overall survival in patients with basal, luminal A and luminal B subtype cancers, with ITM2A primary tumor mRNA levels a positive prognostic indicator for basal and luminal A subtype patients.

Discussion

Invasive breast cancer is a medical problem with a 27% 5-year survival rate for women whose disease has spread to distant sites^{5,6}. To facilitate understanding of the basic transcriptional differences between primary tumors of the breast and the tissues from which these tumors originate, normal breast tissues, we performed comparative transcriptome analysis using two independently published microarray datasets^{2,3}, providing evidence here that differential expression of integral membrane protein 2A, encoded by ITM2A, is a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple negative breast cancer. ITM2A was expressed at significantly lower levels in primary tumors from patients with breast cancer, as compared to normal breast. In patients with basal, luminal A, and luminal B subtype cancer, primary tumor expression of ITM2A was correlated with overall survival. Integral membrane protein 2A and the molecular processes to which it pertains to may be relevant to the initiation or progression of human breast cancer.

References

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Rank: 64
Probe ID: 4013549
p-value: 1.31E-15
t: 1.14E+01
B: 25.326161
Gene: ITM2A
Gene name: integral membrane protein 2A

Chart 1: ITM2A is differentially expressed in the primary tumors of patients with early-onset human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Rank: 2217
Probe ID: 20917
p-value: 7.33E-06
t: 5.0656557
B: 3.452282
Gene: ITM2A
Gene name: integral membrane protein 2A

Chart 2: ITM2A is differentially expressed in the tumor cells of patients with triple-negative breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Figure 1: ITM2A is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of ITM2A in normal breast tissue (left) and in primary tumors of the breast (right) is graphically depicted with the result of a statistical test evaluating significance of difference in ITM2A expression between normal breast tissue and primary tumors of the breast, a p -value, is $p < 0.0001$.

Figure 2: ITM2A expression correlates with overall survival in patients with basal, luminal A, and luminal B subtype breast cancer, but not in patients with HER2+ cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=404$ patients with basal subtype breast cancer (upper left), $n=166$ patients with HER2+ breast cancer (upper right), $n=794$ patients with luminal A breast cancer (lower left), and $n=515$ patients with luminal B breast cancer (lower right), stratified into two groups, based on low or high expression of ITM2A 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 ITM2A 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.

Basal patients

Low ITM2A expression: 39.5 months

High ITM2A expression: 97.48 months

Luminal A patients

Low ITM2A expression: 99.6 months

High ITM2A expression: 169.2 months

Luminal B patients

Low ITM2A expression: 198.44 months

High ITM2A expression: 197.62 months

Chart 3: In basal and luminal A subtype breast cancer, median overall survival is superior in patients with high primary tumor expression of ITM2A.

The overall survival of $n=404$ basal subtype breast cancer patients, $n=794$ luminal A subtype breast cancer patients, and $n=515$ luminal B subtype breast cancer patients, based on stratification into low or high tumor expression of ITM2A is listed in this chart.