Differential expression of mab-21 like 1 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 mab-21 like 1, MAB21L1, when comparing primary tumors of the breast to the tissue of origin, the normal breast. MAB21L1 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. MAB21L1 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 MAB21L1 in primary tumors of the breast was correlated with overall survival in patients with luminal A subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by molecular subtype. MAB21L1 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, MAB21L1, mab-21 like 1, 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. mab-21 like 1 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 in conjunction with GEO2R. 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 MAB21L1 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 MAB21L1 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}.

MAB21L1 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 mab-21 like 1, MAB21L1, 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, MAB21L1 ranked 219 out of 19076 total transcripts, equating to 98.9% differential expression (Chart 1). Differential expression of MAB21L1 in female breast cancer was statistically significant (Chart 1; $p=6.62E-13$).

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 MAB21L1 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

1 compared to normal mammary gland ductal cells, MAB21L1 ranked 5611 out of 45015 total transcripts,
2 equating to 87.5% differential expression (Chart 2). Differential expression of MAB21L1 in the tumor
3 cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=7.47E-04$).
4 These data suggested that differential expression of MAB21L1 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.

5 **MAB21L1 is expressed at significantly lower levels in breast tumors as compared to the breast.**

6 We obtained exact mRNA expression levels for MAB21L1 from the breast and from breast tumors
7 to understand the magnitude and direction of MAB21L1 expression change. MAB21L1 was expressed at
8 lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression
9 of MAB21L1 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). MAB21L1 was
10 expressed at 6.01 ± 0.44 arbitrary units (AU) in normal breast tissue, while it was expressed at 4.93 ± 0.37
11 AU in tumors of the breast. We calculated a mean fold change of 0.82 in MAB21L1 mRNA levels when
comparing primary tumors of the breast to normal breast tissues.

12 **MAB21L1 expression correlates with survival outcomes in luminal A human breast cancer.**

13 We performed Kaplan-Meier survival analysis in an attempt to correlate MAB21L1 mRNA
14 expression levels with survival outcomes in patients with breast cancer, evaluating correlations between
15 molecular subtype and overall survival. We observed a statistically significant correlation between
16 MAB21L1 expression and overall survival (OS) in patients with luminal A subtype breast cancer (Figure
17 2; log rank p -value: 0.00012 for overall survival, hazard ratio: 0.53 (0.38-0.74) (Fig. 2)). MAB21L1
18 mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. Median
OS was 99 months for luminal A patients with low tumor expression of MAB21L1 while median OS was
191.21 months for luminal A patients with high tumor expression of MAB21L1 (Chart 3). MAB21L1
primary tumor expression was not correlated with overall survival in basal subtype breast cancer (Figure
2; log rank p -value: 0.63 for OS, hazard ratio: 0.91 (0.62-1.33) (Fig. 2)), luminal B subtype breast cancer
(Figure 2; log rank p -value: 0.89 for OS, hazard ratio: 0.98 (0.69-1.38) (Fig. 2)) or in HER2+ breast cancer
(Figure 2; log rank p -value: 0.43 for OS, hazard ratio: 0.8 (0.45-1.41) (Fig. 2)).

20 Thus, through comparative transcriptome analysis of primary tumors of the breast and normal
21 breast tissue, we found that differential expression and down-regulation of MAB21L1 was among the
22 most significant transcriptional features in primary tumors from patients with breast cancer. MAB21L1
expression in primary tumors of the breast was correlated with overall survival in patients with luminal A
subtype disease, with mRNA levels of MAB21L1 a positive prognostic indicator for luminal A breast
cancer patients.

23 **Discussion**

24 Invasive breast cancer is a medical problem with a 27% 5-year survival rate for women whose
25 disease has spread to distant sites^{5,6}. To facilitate understanding of the basic transcriptional differences
26 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
27 datasets^{2,3}, providing evidence here that differential expression of mab-21 like 1, encoded by MAB21L1, is
a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple
negative breast cancer. MAB21L1 was expressed at significantly lower levels in primary tumors from
patients with breast cancer as compared to normal breast tissue. Importantly, in patients with luminal A
28 subtype breast cancers, expression of MAB21L1 was correlated with overall survival. Mab-21 like 1

1 type B and the molecular processes to which it pertains to may be relevant to the initiation or progression
2 of human breast cancer.

References

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Rank: 219
Probe ID: 3509411
p-value: 6.62E-13
t: 9.52
B: 19.199582
Gene: MAB21L1
Gene name: mab-21 like 1

Chart 1: MAB21L1 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: 5611
Probe ID: 25121
p-value: 7.47E-04
t: 3.6174121
B: -0.975383
Gene: MAB21L1
Gene name: MAB21L1

Chart 2: MAB21L1 is differentially expressed in the primary 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: MAB21L1 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of MAB21L1 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 MAB21L1 expression between normal breast tissue and primary tumors of the breast, a *p*-value, is $p<0.0001$.

Figure 2: MAB21L1 expression correlates with overall survival in patients with luminal A subtype breast cancer, but not in patients with basal subtype, luminal B subtype, or HER2+ subtype 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 MAB21L1 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 MAB21L1 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.

Luminal A patients

Low MAB21L1 expression: 99 months

High MAB21L1 expression: 191.21 months

Chart 3: In luminal A subtype breast cancer, median overall survival is superior in patients with high primary tumor expression of MAB21L1.

The overall survival of $n=794$ luminal A subtype breast cancer patients based on stratification into low or high tumor expression of MAB21L1 is listed in this chart.