Expression of Phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1) / Noxa correlates with survival in primary and metastatic human breast cancer.

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Women diagnosed with stage I breast cancer have a 99% 5-year survival rate while women diagnosed with stage IV metastatic breast cancer that has spread beyond the breast are presented with a 27% 5-year survival rate (1). There are limited treatment options available for women diagnosed with metastatic breast cancer. Understanding how gene expression differs in patients that are longer survivors of breast cancer can facilitate target discovery for novel therapeutics. In this study we compared the transcriptomes of primary tumors from patients with breast cancer based on survival at 24 months and identified phorbol-12-myristate-13-acetate-induced protein 1 (2), PMAIP1, also known as Noxa (3) as among the most differentially expressed genes the tumors of women that survived past 24 months versus those that expired before 24 months. Transcriptome comparison using an independent dataset, this from the metastatic tissues of patients with stage IV breast cancer again identified PMAIP1/Noxa as differentially expressed based on survival at 24 months (4). In both cases, PMAIP1 was expressed at significantly higher levels in the tumors of survivors. PMAIP1 is member of the BCL-2 family of proteins that regulate apoptosis, a type of cell death (5, 6). These data demonstrate that PMAIP1 expression is positively correlated with survival and together with the molecular function of PMAIP1 suggest that increased PMAIP1 expression in the tumors of patients with enhanced survival of metastatic breast cancer might function to promote cell death of tumor cells. Modulation of PMAIP1 expression may be a relevant therapeutic strategy in patients with breast cancer.

Keywords: breast cancer, metastatic breast cancer, PMAIP1, Noxa, phorbol-12-myristate-13-acetate-induced protein 1, targeted therapeutics in breast cancer, tumor transcriptome analysis, systems biology of breast cancer.

Each year in the United States over a quarter of million women are diagnosed with breast cancer and over 42,000 women will die after being diagnosed with the disease (8). 12% of women in the United States will be diagnosed with breast cancer at some point in their lifetime (9). Patients diagnosed with the more advanced form of the disease, stage IV metastatic breast cancer, have limited treatment options (9). Development of treatment strategies can be facilitated by the specific identification of therapeutic targets as this can both increase efficacy of the treatment and reduce “side-effects” that are actually unwanted effects of a treatment that lacks specificity. In order to facilitate discovery of therapeutic targets in patients with breast cancer, including patients with metastatic breast cancer, we mined published microarray datasets generated using tumor tissues from patients with breast cancer (2, 4). By comparing tumor transcriptomes from patients with metastatic breast cancer based on overall survival of the patient at 24 months, we identified the phorbol-12-myristate-13-acetate-induced protein 1, PMAIP1, also known as Noxa as among the genes whose expression most significantly changed in the metastatic tumors of those surviving past 24 months versus those who expired before 24 months (4). Analysis of a separate dataset from the primary tumors of patients with breast cancer revealed that PMAIP1/Noxa was similarly differentially expressed in primary breast cancer based when comparing tumor transcriptomes based on disease-free survival at 24 months (2). PMAIP1 is a pro-apoptotic member of the BCL-2 family of proteins that regulate apoptosis (5, 7). We found PMAIP1 was expressed at significantly higher levels in the tumors of patients with disease-free (2) or overall survival (4) of localized and metastatic breast cancers, respectively, which suggests that increased expression of PMAIP1 functions to enhance cell death in the tumors of patients with breast cancer translating to enhanced survival for the patient. PMAIP1 may be a target of interest for therapeutic intervention in metastatic breast cancer.

Methods

GSE48391 (2) was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with n=70 for primary tumors of the breast from women with disease-free survival greater than 24 months and an n=11 for primary tumors of the breast from women with disease-free survival less than 24 months.

GSE124647 (4) was generated using Affymetrix Human Genome U133 Array from biopsies from patients with stage IV metastatic breast cancer with an n=70 for metastatic tumor tissues from women surviving greater than 24 months and an n=70 for metastatic tumor tissues from women surviving less than 24 months.

The Benjamin and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of 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 the significance of difference between mRNA expression levels of PMAIP1 in the primary tumors of women surviving less than 24 months as compared to the primary tumors of women surviving greater than 24 months, as well as in the metastatic tissues of women surviving less than 24 months as compared to the metastatic tissue of women surviving greater than 24 months using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We performed an unbiased, systems-level comparative transcriptome analysis based on survival outcomes at 24 months to discover genes associated with enhanced survival in breast cancer using two independent published datasets (2, 4).

PMAIP1/Noxa is differentially expressed in the primary tumors of patients with breast cancer when comparing tumor transcriptomes based on disease-free survival at 24 months

We identified PMAIP1 as differentially expressed in the primary tumors of patients with breast cancer with disease-free survival greater than 24 months as compared to the primary tumors of patients with breast cancer with disease-free survival less than 24 months (2). When ranking all of the transcripts produced in the primary tumors of patients with breast cancer based on change in expression between patients with disease-free survival greater or less than 24 months, PMAIP1 ranked 163 out of 54674 transcripts. Differential expression of PMAIP1 in the tumors of patients with disease-free survival greater than 24 months was statistically significant (Table 1; $p=0.00169882$)

PMAIP1/Noxa is differentially expressed in the metastatic tumors of patients with breast cancer when comparing tumor transcriptomes based on overall survival at 24 months

We also identified PMAIP1 as differentially expressed in the metastatic tumor tissues of patients with breast cancer who survived greater than 24 months (overall survival) as compared to the metastatic tumor tissues of patients with breast cancer who survived less than 24 months (4). When ranking all of the transcripts produced in the metastatic tumor tissues of patients with breast cancer based on change in expression between patients possessing overall survival greater or less than 24 months, PMAIP1 ranked 116 out of 22283 transcripts. Differential expression of PMAIP1 in the tumors of patients with overall survival greater than 24 months was statistically significant (Table 1; $p=4.1E-04$).

PMAIP1 is expressed at significantly higher levels in the primary breast tumors of patients with disease-free survival greater than 24 months

Next, we extracted exact mRNA expression levels from the primary breast tumors of patients with disease-free survival greater or less than 24 months, in order to compare expression levels of PMAIP1 between these two groups as opposed to relative to the entire transcriptome. PMAIP1 was expressed at higher levels in the primary breast tumors of patients with disease-free survival greater than 24 months, and this difference was statistically significant (Figure 1; $p=0.0074$)

PMAIP1 is expressed at significantly higher levels in the metastatic tumor tissues of patients with stage IV breast cancer that survive greater than 24 months

We also extracted exact mRNA expression levels from the metastatic tumor tissues of stage IV metastatic breast cancer patients with overall survival greater or less than 24 months, in order to compare expression levels of PMAIP1 between these two groups as opposed to relative to the entire transcriptome. PMAIP1 was expressed at higher levels in the metastatic tumor tissues of patients with overall survival greater than 24 months, and this difference was also statistically significant (Figure 2; $p=0.0005$).

Thus, we found that PMAIP1/Noxa was among the genes most differentially expressed based on survival outcomes at 24 months in primary and metastatic tumor tissues from patients with breast cancer, and that PMAIP1/Noxa was expressed at significantly higher levels in the tumor tissues of patients with greater survival outcomes.

Discussion

Breast cancer is a leading cause of death for women (8). 12% of women will be diagnosed with breast cancer over the course of their lifetime in the United States (9). We used a systems-level approach to identify genes associated with enhanced survival outcomes in patients with breast cancer by comparing the tumor transcriptomes of primary and metastatic tumor tissues based on survival outcomes at 24 months. For our comparative transcriptome analysis of primary tumors from patients with breast cancer, survival outcomes were disease-free survival. For our comparative transcriptome analysis of metastatic tumor tissues isolated from patients with stage IV metastatic breast cancer, survival outcomes were overall survival. In both cases, we identified the phorbol-12-myristate-13-acetate-induced protein, PMAIP1, also known as NOXA as differentially expressed in patients with breast cancer based on survival outcomes at 24 months. PMAIP1 was expressed at significantly higher levels in the tumor tissues of patients with disease-free survival and overall survival greater than 24 months.

1 These data suggest PMAIP1 expression is correlated with enhanced survival outcomes in
2 patients with breast cancer.

3 PMAIP is a member of the BCL-2 family of apoptotic regulators that include both anti-
4 apoptotic proteins and pro-apoptotic proteins (5, 7). PMAIP1 has pro-apoptotic functions that
5 intersect with the p53 pathway (10-12). p53 is known as the guardian of the genome because
6 of its integral role in sensing and responding to events that are associated with DNA damage
7 by inducing cell cycle arrest to prevent that cell from acquiring a mutation in its genetic material
8 and subsequently dividing, thus spreading this mutation to daughter clones (10). If any of
9 these mutations affect pathways related to proliferation, apoptosis, the cell cycle, a tumor
10 suppressor, or any number of other pathways linked with cell survival and normal growth
11 behaviors, these acquired mutations will serve as a trigger to drive tumor growth (10). By
12 intersecting with the p53 pathway, PMAIP1 is able to connect the mitochondrial apoptotic
13 pathway with p53's function in guarding the genome and triggering cell death upon sensing of
14 any such mutagenic event (10-12). Specifically, the function of BCL-2 proteins (13) and of
15 Noxa (14, 15) are related to the membrane potential and integrity of an intact mitochondrial
16 outer membrane. Depolarization of the mitochondrial membrane by pore formation induces
17 release of cytochrome C which is central to the apoptotic cell death signaling pathway by
18 binding of Apaf-1, the apoptotic protease activating factor 1 (16). Apaf-1 in conjunction with
19 cytochrome C and dATP form the apoptosome, a molecular platform for the activation of the
20 initiator caspase, caspase 9 (17). Activation of caspase 9 by Apaf-1 enables it to cleave
21 downstream caspases including caspase 3 and caspase 7 which go on to effect the apoptotic
22 cell death pathway (18-20). Thus, PMAIP1/Noxa connects the apoptotic cell death pathway
23 orchestrated by mitochondrial outer membrane dynamics to sensing of cellular insults by p53.

26 With respect to known roles for PMAIP1/Noxa in cancer, the PMAIP1 gene was
27 identified as a tumor suppressor gene in pancreatic cancer (21). In acute lymphoblastic
28 leukemia cells, decreased expression of PMAIP1 was associated with impaired sensitivity of
ALL cancer cells to glucocorticoids, used in the treatment of ALL (22). Genetic studies

1 revealed that a variant proximal to the PMAIP1 gene was associated with increased sensitivity
2 in healthy individuals to bleomycin, another drug used in chemotherapy (23). A study of mantle
3 cell lymphoma (MCL) has revealed an interesting discrepancy between RNA message levels
4 and protein levels of PMAIP1 as MCL cells express relatively high levels of Noxa mRNA but
5 relatively low levels of Noxa protein (24). Small molecules that caused accumulation of Noxa
6 like bortezomib lead to death of MCL cells which was dependent on Noxa (24).

7
8 We found in this study that PMAIP1/Noxa was among the genes whose expression was
9 most different between the tumors of patients with breast cancer, both localized and metastatic
10 (2, 4) when comparing tumor transcriptomes based on disease-free survival (2) or overall
11 survival (4) at 24 months, respectively. PMAIP1 has functions in the induction of apoptotic cell
12 death that intersect with p53. We found that the tumors of patients with disease-free survival
13 or overall survival greater than 24 months expressed increased levels of PMAIP1/Noxa mRNA
14 compared to those with survival outcomes less than 24 months. Thus, we found that PMAIP1
15 expression in patient tumors is associated with enhanced survival outcomes in metastatic
16 breast cancer. Modulation of PMAIP1/Noxa expression may be a relevant therapeutic strategy
17 in patients with metastatic breast cancer.
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Rank	ID	p-value	t	B	Gene	Gene name
163	204286_s_at	0.00169882	3.24	-1.52822	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1

Table 1: PMAIP1 is differentially expressed in the primary tumors of patients with breast cancer based on disease-free survival at 24 months.

The rank of differential expression globally, out of 54674 transcripts in total, the probe ID, the p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the gene and gene name are all listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
116	204285_s_at	4.1E-04	3.61984768	-0.07392	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1

Table 2: PMAIP1 is differentially expressed in the metastatic tumor tissues of patients with breast cancer based on overall survival at 24 months.

The rank of differential expression globally, out of 22283 transcripts in total, the probe ID, the p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the gene and gene name are all listed in this chart.

Figure 1: PMAIP1 is expressed at significantly higher levels in the primary tumors of breast cancer patients with disease-free survival greater than 24 months.

The expression of PMAIP1 in the primary tumors of patients with breast cancer based on disease-free survival at 24 months is shown on the left by patient with the mean value marked and p-value listed, and on the right as a violin plot with the median value, 25th and 75th percentiles marked and p-value listed.

Figure 2: PMAIP1 is expressed at significantly higher levels in the metastatic tumors of breast cancer patients with overall survival greater than 24 months.

The expression of PMAIP1 in the metastatic tumor tissues of patients with breast cancer based on overall survival at 24 months is shown on the left by patient with the mean value marked and p-value listed, and on the right as a violin plot with the median value, 25th and 75th percentiles marked and p-value listed.