MAP4K2 expression associates with survival in triple negative breast cancer.

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We mined published microarray data (1) to understand the most significant gene expression differences in the tumors of triple negative breast cancer patients based on survival following treatment: dead or alive. We observed significant transcriptome-wide differential expression of mitogen-activated protein kinase kinase kinase 2, encoded by MAP4K2 when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, MAP4K2 expression was correlated with distant metastasis-free survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. MAP4K2 may be of relevance as a biomarker or as a molecule of interest in understanding the etiology or progression of triple negative breast cancer.

Keywords: triple negative breast cancer, TNBC, systems biology of breast cancer, targeted therapeutics in breast cancer, MAP4K2, mitogen-activated protein kinase kinase kinase 2.

Triple negative breast cancer is defined by lack of expression of the receptors for the hormones progesterone and estrogen (PR negative and ER negative), as well as for the human epidermal growth factor receptor 2 (HER2 negative) (2, 3). Though not completely identical, triple negative breast cancer shares significant overlap with the basal or basal-like molecular subtype of human breast cancer (3). TNBC is more frequently diagnosed in women of African descent (black women) for reasons not understood, and their disease course is similarly more aggressive than in white women with triple negative breast cancer (2, 3). To understand the transcriptional biology of triple negative breast cancer in an unbiased fashion and at the systems level, we mined published microarray data paired with patient survival data (1), performing comparative transcriptome analysis based on survival at time of data collection: dead or alive. We present here a finding from the results of this analysis.

Methods

We used dataset GSE142102 (1) for this differential gene expression analysis in conjunction with GEO2R. GSE142102 was generated using Affymetrix Human Gene 2.1 ST Array technology; in this analysis, we used data from $n=87$ tumors from TNBC patients that were dead at time of analysis and $n=123$ tumors from TNBC patients that were alive at time of analysis. 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 MAP4K2 expression was significantly different between the primary tumors of TNBC patients that were dead or alive using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool (4) for correlation of MAP4K2 mRNA expression levels with distant metastasis-free survival (DMFS) in $n=630$ patients with basal subtype breast cancer.

Results

We performed comparative transcriptome analysis using published microarray data (1) to understand in an unbiased fashion the most significant gene expression differences in the primary tumors of patients with triple negative breast cancer based on survival outcomes.

MAP4K2 is differentially expressed in triple negative breast cancer and negatively correlates with survival.

When comparing primary tumors of 87 live patients with triple negative breast cancer with that of 123 dead TNBC patients we identified mitogen-activated protein kinase kinase kinase 2, *MAP4K2*, as among the most significant transcriptional differences based on survival in human triple negative breast cancer. When ranking each of the transcripts whose expression was measured by microarray based on significance of difference in expression between the tumors of patients dead and alive, MAP4K2 ranked 50 out of 53617 total transcripts, equivalent to 99.9% differential expression (Chart 1). MAP4K2 differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00116898$).

MAP4K2 is expressed at significantly higher levels in the breast tumors of TNBC patients when comparing based on survival.

We obtained exact mRNA expression levels for MAP4K2 from breast tumors of TNBC patients

1 dead and alive to understand the magnitude and direction of MAP4K2 expression change based on
2 survival outcomes. MAP4K2 was expressed at higher levels in tumors of TNBC patients alive as
3 compared to that of TNBC patients dead (Figure 1). Increased expression of MAP4K2 in primary breast
4 tumors was statistically significant (Figure 1: $p=0.0009$). MAP4K2 was expressed at -0.24 ± 0.92
5 arbitrary units (AU) in the primary tumors of TNBC patients dead, while it was expressed at 0.22 ± 1.02
6 AU in the primary tumors of TNBC patients alive.

7 MAP4K2 expression correlates with distant metastasis-free survival in basal subtype human breast cancer.

8 We referenced a bioinformatics tool containing Kaplan-Meier survival data of human breast cancer
9 patients to understand more thoroughly whether a relationship existed between MAP4K2 primary tumor
10 expression and patient survival in human breast cancer. We found that MAP4K2 expression was
11 correlated with distant metastasis-free survival in patients with basal or basal-like human breast cancer, a
12 breast cancer molecular subtype which shares significant overlap with triple negative breast cancer; this
13 correlation approached statistical significance (Figure 2; log rank p -value: 0.091 for distant metastasis-free
14 survival, hazard ratio: 0.78 (0.58-1.04) (Fig. 2)). MAP4K2 mRNA levels were a positive prognostic
15 indicator in basal subtype breast cancer patients. Median DMFS was 26.99 months for basal subtype
16 patients with low tumor expression of MAP4K2 as compared to 43.44 months for basal subtype patients
17 with high tumor expression of MAP4K2.

18 Thus, we found through unbiased comparative transcriptome analysis that MAP4K2 was among
19 the genes whose expression was most significantly different in the primary tumors of triple negative breast
20 cancer patients alive when compared to that of TNBC patients dead, that MAP4K2 was expressed at
21 significantly higher levels in the primary tumors of TNBC patients alive as compared to TNBC patients
22 dead, and that MAP4K2 expression was positively correlated with distant metastasis-free survival in the
23 basal or basal-like molecular subtype.

24 **Discussion**

25 It is not understood why women of African descent (black women) are diagnosed with triple
26 negative breast cancer more frequently than white women, and why this disease is more aggressive in
27 black women. MAP4K2 may be of relevance as a biomarker in identifying patients who are more likely to
28 survive, perhaps as part of a panel of genes used for prognostic stratification, and the molecule itself may
be important to the biology of triple negative breast cancer etiology or more likely its progression. Blind
systems-level analyses, like this, can help shed light on the fundamental molecular characteristics of a
disease whose racial propensities demand further explanation.

References

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Rank: 50
Probe ID: 16740037
p-value: 0.00116898
t: -3.2463439
B: -3.01
Gene: MAP4K2
Gene name: mitogen-activated protein kinase kinase kinase 2

Chart 1: MAP4K2 is differentially expressed at the transcriptome-level when comparing the primary tumors of TNBC patients dead and alive.

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: MAP4K2 is expressed at significantly higher levels in the primary tumors of TNBC patients alive as compared to the primary tumors of TNBC patients dead.

The mRNA expression level of MAP4K2 in the primary tumors of TNBC patients dead (left) and in primary tumors of TNBC patients alive (right) is graphically depicted with the result of a statistical test evaluating significance of difference in MAP4K2 expression between the primary tumors of TNBC patients dead and alive, a p -value, listed here: $p=0.0009$.

Figure 2: MAP4K2 expression correlates with distant metastasis-free survival in basal subtype human breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival (DMFS) for $n=630$ breast cancer patients of basal subtype, based on low or high expression of MAP4K2 in patient primary tumors. The log rank p-value denoting statistical significance of difference in recurrence-free 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 MAP4K2 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.

1 Low MAP4K2 expression: 26.99 months

2 High MAP4K2 expression: 43.44 months

3
4 **Chart 2: Median distant metastasis-free survival is superior in basal subtype breast cancer patients with high primary tumor expression of MAP4K2.**

5 The median DMFS (distant metastasis-free survival) of basal subtype breast cancer patients, with low
6 primary tumor expression of MAP4K2 and high primary tumor expression of MAP4K2 is listed in this
7 chart.
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