The AP-1 transcription factor is differentially expressed in breast cancer and predictive of disease stage.

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The AP-1 transcription factor is a heterodimer, composed of subunits FOS and JUN (1-5), each of which have paralogs such as FOSB (6) and JUND (7). We probed the transcriptomes of 930 tumors from patients with breast cancer at stages I, II and III to understand how transcription differs based on tumor progression at the systems level and in an unbiased fashion by mining five independent published microarray datasets (8-12). We identified both FOS and JUN as among the genes most differentially expressed when comparing the tumor transcriptomes of patients with breast cancer from stage I, stage II and stage III. We also identified FOSB and JUND as differentially expressed based on cancer stage in the tumors of patients with breast cancer. In particular, expression of the AP-1 subunit FOS was predictive of breast cancer stage, with an overall trend of decreasing FOS expression in more advanced cancer stages. These data point to a negative relationship between AP-1 transcription factor expression and breast cancer stage and illustrate the potential of transcriptome analysis to illuminate fundamental transcriptional differences underlying tumor progression.

Keywords: AP-1, FOS, JUN, JUND, FOSB, breast cancer, staging in breast cancer, systems biology of breast cancer, tumor transcriptome analysis, targeted therapeutics in breast cancer.

Survival in breast cancer decreases as the disease stage increases. Staging has historically utilized size of the tumor, degree of affectedness at the lymph nodes, and presence or absence of distant metastases to classify breast cancer into four stages: I, II, III, and IV, with stage IV being reserved for patients with any level of distant metastasis. While patients with localized, stage I breast cancer have a 99% 5-year survival rate, patients with regional breast cancer including stage II and stage III breast cancers have 86% 5-year survival rates (13, 14). Once the disease stage has reached stage IV, involving distant metastases, the 5-year survival rate for the affected patient has reached 27% (13, 14). We hypothesized that the transcriptional behavior of a primary tumor, independent of patient pathology, might be able to classify tumors into discrete stages. We also sought to determine if we could identify fundamental transcriptional differences between tumors in patients with breast cancer based on disease stage. By probing five independent published microarray datasets (8-12), encompassing 930 tumors in total, we identified the AP-1 transcription factor as significantly differentially expressed in human breast cancer. Subunits FOS, FOSB, JUN and JUND were all found as among the genes whose expression changed most significantly between breast cancers staged I-III. AP-1 expression, and in particular FOS, appeared to negatively correlate with breast cancer stage. The results from these small-scale, preliminary analyses suggest that similar analyses using greater numbers of datasets and in larger cohorts may be able to classify patients into discrete stages based on the transcriptional behavior of the primary tumor.

Methods

We used datasets GSE109169, GSE102484, GSE76275, GSE86166 and GSE124648 for this systems-level analysis of the breast cancer transcriptome based on disease stage using GEO2R. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array with n=25 tumors in total, with n=6 stage I, n=11 stage II, and n=7 stage III tumors from patients with early onset breast cancer. GSE102484 was generated using Affymetrix Human Genome

U133 Plus 2.0 Array with n=677 tumors total, with n= 175 stage I, n= 328 stage II, and n= 174 stage III tumors. GSE76275 was generated using Affymetrix Human Genome U133 Plus 2.0 Array with n=30 tumors in total, with n=10 stage I, n=12 stage II, and n=8 stage III tumors from patients with triple negative breast cancer. GSE86166 was generated using n=78 tumors in total with n=26 stage I, n=28 stage II, n=24 stage III tumors from patients with HER2+ and HER2- breast adenocarcinomas. GSE124648 was generated using Affymetrix Human Genome U133A Array with n=120 tumors in total, with n=20 stage I, n=80 stage II, and n=20 stage III from patients with HR+/HER2-, HR+/HER2+, ER+/HER2-, and ER+/HER2+ breast cancers.

The Benjamini 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 AP-1 transcription factor subunit expression levels in patient tumors based on stage, from stage I-III, using an ordinary one-way ANOVA with Dunnett's multiple comparisons test. 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 mined five independent published microarray datasets generated using the tumors of patients with breast cancer, encompassing 930 tumors in total, to identify genes whose expression was most significantly different between tumor stages I, II and III in human breast cancer. We identified multiple subunits of the AP-1 transcription factor as differentially expressed in the tumors of patients with breast cancer when comparing tumor transcriptomes based on stage.

FOS is differentially expressed in human breast cancers when comparing the transcriptomes of stage I tumors to stage II and stage III tumors.

FOS was the single most differentially expressed gene when comparing the transcriptomes of tumors at stage I, stage II and stage III, out of 54675 transcripts in total. Differential expression of FOS based on tumor stage in human breast cancer was statistically significant (Table 1; $p=3.8E-15$).

In a separate dataset, when sorting all of the genes expressed in patient tumors based on change in expression between tumor stages I, II and III, FOS ranked 28 out of 60607 total transcripts. Differential expression of FOS in this separate dataset of tumors from patients with breast cancer was statistically significant (Table 1; $p=0.00009938$).

FOSB is differentially expressed in human breast cancers when comparing the transcriptomes of stage I tumors to stage II and stage III tumors.

FOS isoform FOSB was also identified as differentially expressed in breast cancer when comparing tumor transcriptomes based on stage. When sorting all of the genes expressed in patient tumors based on change in expression between tumor stages I, II and III, FOSB ranked 125 out of 60607 total transcripts. Differential expression of FOSB was statistically significant (Table 2; $p=0.00074745$).

JUN is differentially expressed in human breast cancers when comparing the transcriptomes of stage I tumors to stage II and stage III tumors.

AP-1 is a heterodimer of FOS and JUN. We identified JUN as differentially expressed in patient tumors in human breast cancer when comparing tumor transcriptomes based on disease stage. When sorting all of the genes expressed in patient tumors based on change in expression between tumor stages I, II and III, JUN ranked 175 out of 22283 total transcripts. Differential expression of JUN was statistically significant (Table 3; $p=0.0074433$).

JUND is differentially expressed in human breast cancers when comparing the transcriptomes of stage I tumors to stage II and stage III tumors.

1 We further identified JUN isoform JUND as differentially expressed in patient tumors in
2 human breast cancer when comparing tumor transcriptomes based on disease stage. When
3 sorting all of the genes expressed in patient tumors based on change in expression between
4 tumor stages I, II and III, JUND ranked 6 out of 19076 total transcripts. Differential
5 expression of JUND was statistically significant (Table 4; $p=0.0008899$).

6 Next, we asked whether AP-1 transcription factor subunit expression was significantly
7 different between tumor stages, by extracting mRNA levels of each AP-1 subunit and
8 comparing their expression by disease stage, in breast cancer stages I, II and III.

10 Expression of FOS in patient tumors significantly decreases as cancer stage progresses.

11 FOS was expressed at significantly lower levels in stage II tumors as compared to stage
12 I tumors (Figure 1A; $p<0.0001$). FOS was expressed at significantly lower levels in stage III
13 tumors as compared to stage I tumors (Figure 1A; $p<0.0001$). FOS was also expressed at
14 significantly lower levels in stage III tumors as compared to stage II tumors (Figure 1A;
15 $p=0.0050$).

16 In a separate dataset, FOS was expressed at significantly lower levels in stage II tumors
17 as compared to stage I tumors (Figure 1B; $p=0.0227$). FOS was expressed at significantly
18 lower levels in stage III tumors as compared to stage I tumors (Figure 1B; $p<0.0001$). FOS was
19 expressed at lower levels in stage III tumors as compared to stage II tumors, but this was not
20 statistically significant (Figure 1B; $p=0.1439$).

23 Expression of FOSB in patient tumors decreases as cancer stage progresses.

24 FOSB was expressed at significantly lower levels in stage II tumors as compared to
25 stage I tumors (Figure 2; $p=0.0023$). FOSB was expressed at significantly lower levels in stage
26 III tumors as compared to stage I tumors (Figure 2; $p=0.0044$). FOSB expression in this
27 dataset was not significantly different when comparing tumors at stage II and tumors at stage
28 III (Figure 2; $p=0.9972$).

1 Expression of JUN in patient tumors based on cancer stage.

2 JUN was expressed at significantly lower levels in stage II tumors as compared to stage
3 I tumors (Figure 3; $p=0.0122$). JUN was expressed at lower levels in stage III tumors as
4 compared to stage I tumors, but this difference was not statistically significant (Figure 3;
5 $p=0.7005$). JUN was expressed at higher levels in stage III tumors as compared to stage II
6 tumors, but this difference was not statistically significant (Figure 3; $p=0.1474$).
7

8
9 Expression of JUND in patient tumors based on cancer stage.

10 JUND was expressed at higher levels in stage II tumors as compared to stage I tumors;
11 this difference approached significance but was not statistically significant (Figure 4;
12 $p=0.0929$). JUND was expressed at lower levels in stage III tumors as compared to stage I
13 tumors, but this difference was not statistically significant (Figure 4; $p=0.2093$). JUND was
14 expressed at significantly lower levels in stage III tumors as compared to stage II tumors
15 (Figure 4; $p=0.0008$).
16

17 Thus, overall, we observed a relationship between AP-1 transcription factor subunit and
18 cancer stage in patients with breast cancer in that more advanced tumors (stage II and stage
19 III) expressed significantly lower levels of FOS and FOSB. The relationship between JUND
20 expression and cancer stage was mixed, and the relationship between JUN expression and
21 cancer stage was statistically significant in the datasets examined only between tumors at
22 stage I and stage II. The relationship between AP-1 transcription factor subunit and cancer
23 stage in the tumors of patients with breast cancer was most clear in the case of FOS.
24
25

26 Expression of the AP-1 transcription factor subunit FOS is predictive of cancer stage in
27 patients with breast cancer.

28 We observed a clear relationship between FOS expression and tumor stage, in that FOS
expression tended to be lower as tumor stage progressed. That conclusion brought us to this

1 question: would a clinician be able to use FOS mRNA levels to predict, or help predict the
2 stage of a patient's tumor? To answer this question, we evaluated whether comparing FOS
3 mRNA levels at the upper and lower bounds of expression based on stage of tumor, in two
4 separate datasets, would reveal a statistically significant relationship between FOS expression
5 and percentage of tumors expressing FOS above or below a certain threshold.

6 In one dataset, FOS expression was lower than 9 in 5.14% of stage I tumors (9/175
7 tumors), 17.68% of stage II tumors (58/328 tumors), and 21.84% of stage III tumors (38/174
8 tumors) (Figure 5A). FOS expression was greater than 12 in 17.14% of stage I tumors (30/175
9 tumors), 5.49% of stage II tumors (18/328 tumors), and 4.02% of stage III tumors (7/174
10 tumors) (Figure 5B). The percentage of tumors expressing FOS at the lowest levels was greater
11 in stage III tumors than in stage II and stage I tumors, and the percentage of tumors expressing
12 FOS at the highest levels was greater in stage I tumors than in stage II and stage III tumors.

14 We performed a similar analyses with a separate dataset. FOS expression was less
15 than 10.5 in 3.85% of stage I tumors (1/26 tumors), 14.29% of stage II tumors (4/28 tumors),
16 and 33.33% of stage III tumors (8/24) (Figure 5C). FOS expression was higher than 12 in
17 15.38% of stage I tumors (4/26 tumors), 3.57% of stage II tumors (1/28 tumors), and 0.00% of
18 stage III tumors (0/24 tumors) (Figure 5D). Again, the percentage of tumors expressing FOS at
19 the lowest levels was greater in stage III tumors than in stage II and stage I tumors, and the
20 percentage of tumors expressing FOS at the highest levels was greater in stage I tumors than
21 in stage II and stage III tumors.

23 In the aggregate, combining data from both analyses, the percentage of tumors
24 expressing FOS at the lowest levels was significantly higher in stage III tumors as compared to
25 stage I tumors (Figure 6A, $p=0.0368$), and the percentage of tumors expressing FOS at the
26 highest levels was significantly lower in stage II (Figure 6B; $p=0.0189$) and stage III tumors
27 (Figure 6B; $p=0.0109$) as compared to tumors from patients with stage I disease.

28 Thus, we observed a statistically significant relationship between FOS mRNA levels and
the tumor stage in patients with breast cancer. This was evident when comparing the whole

transcriptomes of patient tumors and asking which genes changed in expression most significantly, as well as when evaluating what percentage of tumors expressed the highest and lowest levels of FOS and assessing whether this percentage was significantly different by disease stage. We conclude that FOS mRNA levels are predictive of tumor stage in patients with breast cancer.

Discussion

Survival outcomes for patients with breast cancer are increasingly dismal as disease stage progresses (13, 14). To understand how the fundamental transcriptional behavior of tumors in human breast cancer differs based on disease stage, and to assess whether gene expression could be used to help predict or classify cancer stage in breast cancer, we used systems-level comparative transcriptome analysis to identify genes whose expression was most different between tumors from patients with breast cancer at stage I, stage II and stage III of disease. We used five independent published microarray datasets for this analysis, encompassing 930 tumors in total (8-12). We identified AP-1 transcription factor subunits FOS, JUN, FOSB and JUND as among the genes whose expression changed most significantly between tumors staged I through III in patients with breast cancer.

There is a wide body of literature regarding the role of AP-1 in tumorigenesis (15, 16) and specifically in breast cancer (17-23). AP-1 is a critical regulator of mammary gland development in mammals (24). Our findings suggest that FOS expression may be useful for prediction of tumor stage in humans with breast cancer. These data also suggest that in general, AP-1 activity may decrease with tumor progression, but it is difficult to infer activity from gene expression data. AP-1 is a central signal transduction pathway with a multitude of inputs from extracellular cues and cues from within the cell, so any translation of these findings to specific signaling pathways must be individually assessed in the laboratory (25).

In general, we found that multiple subunits of the AP-1 transcription factor are differentially expressed when comparing tumors in human breast cancer based on disease

stage, and that FOS expression was significantly correlated with disease stage. Stage I tumors expressed significantly higher levels of FOS than stage II and stage III tumors, and FOS expression levels at the highest and lowest levels were useful as one criteria in predicting cancer stage. These data illustrate the potential for systems-level investigations of gene expression using tumor transcriptome data for patient stratification and for the understanding of fundamental transcriptional differences in disease that accompany tumor progression.

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Rank	ID	p-value	F	Gene	Gene name
GSE102484					
1	209189_at	3.8E-15	34.8894642	FOS	Fos proto-oncogene, AP-1 transcription factor subunit
GSE86166					
28	merck-NM_005252_at/ NM_005252	0.00009938	1.04E+01	FOS	Fos proto-oncogene, AP-1 transcription factor subunit

Table 1: The AP-1 transcription factor subunit FOS is differentially expressed in human breast cancers based on disease stage, when comparing the transcriptomes of stage I, stage II and stage III tumors.

The rank of differential expression, probe/transcript ID, the p-value of global differential expression, the F statistic, the gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene
GSE86166				
125	merck2- BX538117_at/ NM_006732 NM_001114171	0.00074745	7.91	FOSB

Table 2: The AP-1 transcription factor subunit FOSB is differentially expressed in human breast cancers based on disease stage, when comparing the transcriptomes of stage I, stage II and stage III tumors.

The rank of differential expression, probe/transcript ID, the p-value of global differential expression, the F statistic, the gene and gene name are listed in this chart.

GSE124648	ID	p-value	F	Gene	Gene name
175	201464_x_at	0.0074433	5.1061012	JUN	Jun proto-oncogene, AP-1 transcription factor subunit

Table 3: The AP-1 transcription factor subunit JUN is differentially expressed in human breast cancers based on disease stage, when comparing the transcriptomes of stage I, stage II and stage III tumors.

The rank of differential expression, probe/transcript ID, the p-value of global differential expression, the F statistic, the gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
GSE109169					
6	3854877NM_005354	0.0008899	9.5901814	JUND	jun D proto-oncogene

Table 4: The AP-1 transcription factor subunit JUND is differentially expressed in human breast cancers based on disease stage, when comparing the transcriptomes of stage I, stage II and stage III tumors.

The rank of differential expression, probe/transcript ID, the p-value of global differential expression, the F statistic, the gene and gene name are listed in this chart.

Figure 1: Expression of FOS in patient tumors significantly decreases as breast cancer stage progresses.

The mRNA expression level of FOS is represented individually by patient with mean value marked (left) and by box and whisker plot from 10-90% with median value marked (right). The result of a statistical test evaluating the significance of difference in expression of FOS compared to stage I tumors, is listed above stage II and stage III tumor data. The p-value comparing expression between stage II and stage III tumors is listed above the indicated line.

Figure 2: Expression of FOSB in patient tumors significantly decreases as breast cancer stage progresses.

The mRNA expression level of FOSB is represented individually by patient with mean value marked (left) and by box and whisker plot from 10-90% with median value marked (right). The result of a statistical test evaluating the significance of difference in expression of FOSB compared to stage I tumors, is listed above stage II and stage III tumor data. The p-value comparing expression between stage II and stage III tumors is listed above the indicated line.

Figure 3: JUN expression in stage II breast cancers is significantly lower than in stage I breast cancers.

The mRNA expression level of JUN is represented individually by patient with mean value marked (left) and by box and whisker plot from 10-90% with median value marked (right). The result of a statistical test evaluating the significance of difference in expression of JUN compared to stage I tumors, is listed above stage II and stage III tumor data. The p-value comparing expression between stage II and stage III tumors is listed above the indicated line.

Figure 4: JUND expression in stage III breast cancers is significantly lower than in stage II breast cancers.

The mRNA expression level of JUND is represented individually by patient with mean value marked (left) and by box and whisker plot from 10-90% with median value marked (right). The result of a statistical test evaluating the significance of difference in expression of JUND compared to stage I tumors, is listed above stage II and stage III tumor data. The p-value comparing expression between stage II and stage III tumors is listed above the indicated line.

Figure 5: Expression of the AP-1 transcription factor subunit FOS is predictive of cancer stage in patients with breast cancer.

The number of patients per tumors stage expressing FOS mRNA levels above and below designated thresholds is graphically represented with FOS mRNA levels on the Y axis.

A

FOS Expression by Stage: Lower Bound

Figure 6: Expression of the AP-1 transcription factor subunit FOS is predictive of cancer stage in patients with breast cancer.

The percentage of stage I, stage II and stage III tumors from patients with breast cancer expressing the lowest (A) and highest (B) levels of FOS are represented with standard error of the mean. The result of a statistical test evaluating the significance of difference in percentage of tumors within each stage, as compared to stage I, is listed above stage II and stage III tumor data. The p-value comparing expression between stage II and stage III tumors is listed above the indicated line.

B

FOS Expression by Stage: Upper Bound