Gene expression of the Interleukin 6 signal transducer, IL6ST (gp130) associates with survival outcomes in breast cancer.

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Breast cancer is a leading cause of death for women in the United States (1). The central reason women die from breast cancer is metastasis, or spread of cancer from the breast to foreign sites such as the bones, liver, brain and lungs (2). We integrated independent microarray datasets, one containing transcriptome data from primary tumors of the breast (3), and the other containing transcriptome data from metastases from women with breast cancer (4), each of which possess survival data associated with the patient from which each tumor or metastasis sample was obtained. By comparing tumor transcriptome data based on survival outcomes, we found that gene encoding the Interleukin 6 signal transducer, IL6ST, gp130 (5), was among the genes whose expression was most different between patients who survived greater or less than 18 months. In both primary tumors of the breast and in metastases, IL6ST was expressed at significantly higher levels in women who survived greater than 18 months. These data are consistent with a role for IL6ST as a biomarker, or prognostic indicator (6-8) to help predict which women can benefit from more or less intensive treatment regimens based on prediction of metastasis; moreover, IL6ST may be fundamentally linked with tumor progression and tumor aggressiveness highlighting potential for IL6ST as a therapeutic target in breast cancer.

Keywords: IL6ST, IL-6 signal transducer, gp130, breast cancer, breast cancer metastasis, systems biology of breast cancer, targeted therapeutics in breast cancer, tumor transcriptome analysis.

Each year in this country, over 42,000 women will die after being diagnosed with breast cancer (1). The vast majority of these deaths are the result of metastasis, the process by which tumors localized to the breast, known as primary tumors, spread beyond local tissue to the lymph nodes, or to more distant foreign sites such as the brain, the lungs, the liver and the bones (2). While the 5-year survival rate for women with breast cancer is 99%, the 5-year survival rate for women with metastatic breast cancer is 27% (9). Enhanced understanding of the fundamental biology that governs the metastatic process is required for development of novel and targeted therapeutics that can halt, delay or reverse metastasis.

In this study, we used a systems-level approach to mine published datasets (3, 4) and discover specific genes whose expression changed most significantly based on the survival outcome of the patient from which the tumor sample was derived. We identified the interleukin-6 signal transducer, IL6ST as one of the genes whose expression was most different in the primary tumors of women with disease-free survival less than 18 months when compared to the primary tumors of women with disease-free survival greater than 18 months (3). We interrogated a separate dataset (4), this containing similar paired survival and transcriptome data, but from metastatic tissues obtained from women with breast cancer that had spread beyond the breast. This analysis again identified IL6ST as among the genes whose expression was most different between the metastases of women who survived the disease less than 18 months and the metastases of women who survived the disease greater than 18 months. IL6ST is utilized as a biomarker to predict metastasis or “distant recurrence” as well as response to endocrine therapy in breast cancers and is included in a clinically available biomarker test (Endopredict) (6-8); this is the first report of survival outcome-associated differential expression of IL6ST in primary as well as metastatic breast cancer.

Methods

We used GSE48391 and GSE124647 for this analysis in conjunction with GEO2R.

1 GSE48391 (3) was generated using Affymetrix Human Genome U133 Plus 2.0 Array
2 technology with n=74 for primary tumors of the breast from women with disease-free survival
3 greater than 18 months and an n=7 for primary tumors of the breast from women with disease-
4 free survival less than 18 months.

5 GSE124647 (4) was generated using Affymetrix Human Genome U133 Array from
6 biopsies from patients with stage IV metastatic breast cancer with an n=52 for metastatic
7 tumor tissues from women surviving greater than 18 months and an n=88 for metastatic tumor
8 tissues from women surviving less than 18 months.

9
10 The Benjamin and Hochberg method of p-value adjustment was used for ranking of
11 differential expression but raw p-values were used for assessment of statistical significance of
12 global differential expression. Log-transformation of data was auto-detected, and the NCBI
13 generated category of platform annotation was used.

14
15 A statistical test was performed to evaluate the significance of difference between mRNA
16 expression levels of IL6ST in the primary tumors of women surviving less than 18 months as
17 compared to the primary tumors of women surviving greater than 18 months, as well as in the
18 metastatic tissues of women surviving less than 18 months as compared to the metastatic
19 tissue of women surviving greater than 18 months using a two-tailed, unpaired t-test with
20 Welch's correction. Only p-values less than 0.05 were considered statistically significant. We
21 used PRISM for all statistical analyses (Version 8.4.0)(455).

22 **Results**

23
24 We performed global comparative differential gene expression analysis to identify
25 specific genes whose expression was associated with increased survival or death in women
26 with primary and metastatic breast cancer using two independently published datasets (3, 4).

27
28 IL6ST is differentially expressed in the primary tumors of women with breast cancer when
stratifying tumor transcriptome data based on survival of the patient.

1 We identified gp130, also known as the interleukin-6 signal transducer, IL6ST (5) as
2 among the genes whose expression was most significantly different when comparing the
3 global gene expression profiles of primary tumors from women with breast cancer surviving
4 less than 18 months with that of primary tumors from women with breast cancer surviving
5 greater than 18 months (3). When sorting each of the transcripts detected in the microarray
6 dataset analyzed here based on magnitude of change in the tumors of women surviving greater
7 or less than 18 months, IL6ST ranked 94 out of 54674 transcripts. Differential expression of
8 IL6ST was statistically significant (Table 1; $p = 2E-03$).
9

10 Using a separate dataset (4), we compared the global gene expression profiles of
11 metastatic tissues from women with breast cancer based on overall survival greater or less
12 than 18 months, and again identified IL6ST as one of the genes whose expression changed
13 most significantly based on survival of the patient from which the metastatic tissue was
14 obtained. When sorting each of the transcripts detected in the microarray dataset analyzed
15 here based on magnitude of change in the metastatic tumor tissue of women surviving greater
16 or less than 18 months, transcripts encoded by the IL6ST gene ranked 1 and 2 out of 22283
17 transcripts. In other words, IL6ST was the single most differentially expressed gene in the
18 metastatic tumor tissues of patients with stage IV metastatic breast cancer based on overall
19 survival at 18 months. Differential expression of IL6ST in metastatic tissue samples was again
20 statistically significant (Table 2; $p = 4.66E-07$ and $4.85E-07$, respectively).
21

22
23 IL6ST is expressed at significantly higher levels in both primary tumors and metastatic tissue
24 from women with breast cancer who survive greater than 18 months.

25 Next, we extracted exact mRNA expression values for IL6ST in the primary tumors of
26 women whose transcriptomes we compared based on disease-free survival at 18 months. We
27 also performed a statistical test to evaluate whether the difference in expression of IL6ST was
28 statistically significant. IL6ST was expressed at higher levels in the primary breast tumors of
patients surviving greater than 18 months. The difference in expression of IL6ST between the

primary tumors of patients surviving more or less than 18 months approached the level of statistical significance (Figure 1; $p=0.0575$).

We also extracted exact mRNA expression values for IL6ST in the metastatic tumor transcriptomes of women and compared these values based on disease-free survival at 18 month. We also performed a statistical test to evaluate whether the difference in expression of IL6ST based on survival at 18 months was statistically significant. IL6ST expression was higher in the metastatic tumor tissues of patients surviving greater than 18 months than in the metastatic tumor tissues of patients surviving less than 18 months. This difference in IL6ST expression was statistically significant (Figure 2; $p<0.0001$).

Thus, we found that IL6ST was expressed at higher levels in both the primary and metastatic tumor tissues of patients with breast cancer who survived greater than 18 months when compared to that of patients surviving less than 18 months. In the metastatic tumor tissues, represented from the lungs, the liver, the bones and soft tissues, this difference in IL6ST expression was statistically significant. These data indicate that IL6ST expression supports survival in patients with breast cancer and suggests that IL6ST expression is protective in these patients.

Discussion

Women diagnosed with metastatic breast cancer have limited treatment options. In this study we sought to identify genes expressed most differently between the tumors of women with breast cancer, both primary and metastatic, based on disease-free or overall survival at 18 months, respectively. These analyses identified the IL-6 signal transducer IL6ST as among the most differentially expressed genes when comparing tumor transcriptomes based on survival at 18 months, with IL6ST being expressed at significantly higher levels in both primary tumors and metastatic tumor tissues of women surviving greater than 18 months.

IL6ST, also known as gp130 is a component of the high affinity IL-6 receptor and is a membrane glycoprotein with six fibronectin type III domains (5). Ectopically expressed IL6ST

1 can associate with soluble IL-6 receptor (IL-6R) and IL-6 to transduce cytokine signals at the
2 cell membrane (5). Indeed the IL-6 family of cytokines, including IL-6, IL-11 and IL-27 are
3 defined by their common usage of gp130 as part of their receptor signaling complexes (10).
4 This family includes the leukemia inhibitory factor (LIF) and oncostatin M (OSM) (10). IL-6 itself
5 has been studied to some extent in breast cancers (11, 12), but with confounding results as to
6 a pro- or anti-tumorigenic role for IL-6 signaling, as *in vitro* studies found IL-6 inhibited the
7 proliferation of breast cancer cells that were estrogen receptor positive while higher expression
8 of IL-6 in the blood of patients with breast cancer indicated a poorer prognosis (12). This was
9 reconciled as IL-6 possessing distinct roles locally, with a general tumor suppressive role for
10 IL-6 within tumors but with IL-6 expression systemically being a marker of tumor burden (12).
11 However, in a study of triple negative breast cancers, investigators came to a more definitive
12 conclusion regarding the role of IL-6, finding that IL-6 was expressed widely in multiple triple
13 negative breast cancer cell lines, with dual inhibition of IL-6 and IL-8 resulting in decreased
14 colony formation and breast cancer cell survival *in vitro* (13). Higher median expression of IL-6
15 was found to correlate with poorer prognosis in patients with triple negative breast cancer and
16 *in vivo*, depletion of IL-6 in conjunction with IL-8 blocked tumor growth (13). IL-6 has also been
17 associated with multi-drug resistance in breast cancer cells through autocrine signaling and
18 induction of the multi-drug resistance gene *mdr1* (14).

21 There is limited information available regarding IL6ST in breast cancers (15-19). One
22 recent report documented expression of full-length IL6ST in three breast cancer cell lines,
23 MCF-7, T47D, and ZR-75-1 (15). Another study reported that IL6ST expression could predict
24 which patients with estrogen receptor positive (ER+) ductal carcinoma in situ (DCIS) might
25 benefit from endocrine therapy as an adjunctive treatment to surgery (16). A third study
26 reported that IL6ST expression was able to successfully predict response to endocrine therapy
27 (ET), concluding that expression of full-length, membrane-bound IL6ST rather than soluble
28 IL6ST was the best predictor of response to ET (17). A fourth study that reported differential
expression of the microRNA miR-188-5p in breast cancers concluded that miR-188-5p

1 functioned in a tumor-suppressive role in breast cancer, finding that *in vitro* miR-188-5p
2 inhibited proliferation and migration through one of its target genes, IL6ST (18). Interestingly,
3 IL6ST has been reported to be expressed at higher levels in males with breast carcinoma as
4 opposed to females (19).

5 We found that the primary breast tumors of women diagnosed with a wide variety of
6 breast cancer types with disease-free survival greater than 18 months expressed higher levels
7 of IL6ST than the primary breast tumors of women with disease-free survival less than 18
8 months. We also found that IL6ST was the most differentially expressed gene when comparing
9 metastatic tumors from women with stage IV metastatic breast cancer surviving greater than
10 18 months to that of metastatic tumors from women expiring before 18 months. These data
11 are suggestive of a protective role for IL6ST in human breast cancers but must be validated in
12 separate cohorts, at the protein level, and in experimental models. It is important to note that
13 one study has documented highly deficient seeding capacity of IL6ST-depleted MDA231-S1a-
14 derived circulating tumor cells in a mouse xenograft model of metastatic breast cancer (20).
15 These data must be reconciled with our unbiased, systems-level findings from the primary and
16 metastatic tumors of humans with breast cancer (3, 4) that argue higher expression of IL6ST
17 correlates with enhanced survival. We identified IL6ST as the single gene whose expression
18 was most quantitatively different in the metastatic tissues of women when comparing tumor
19 transcriptomes based on survival at 18 months, expressed at significantly higher levels in the
20 metastatic tissues of women surviving stage IV metastatic breast cancer greater than 18
21 months.
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Rank	ID	p-value	t	B	Gene	Gene name
94	212195_at	2E-03	3.19	-3.24	IL6ST	interleukin 6 signal transducer

Table 1: IL6ST is differentially expressed in the primary tumors of patients with breast cancer based on disease-free survival at 18 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
1	212195_at	4.66E-07	5.2845293	5.9333	IL6ST	interleukin 6 signal transducer
2	204863_s_at	4.85E-07	5.2756696	5.89718	IL6ST	interleukin 6 signal transducer

Table 2: IL6ST is differentially expressed in the metastatic tumor tissues of patients with breast cancer based on overall survival at 18 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: IL6ST is expressed at higher levels in the primary tumors of breast cancer patients with disease-free survival greater than 18 months.

The expression of IL6ST in the primary tumors of patients with breast cancer based on disease-free survival at 18 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: IL6ST is expressed at significantly higher levels in the metastatic tumors of breast cancer patients with overall survival greater than 18 months.

The expression of IL6ST in the metastatic tumor tissues of patients with breast cancer based on overall survival at 18 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.