Bevacizumab treatment of patients with breast cancer is associated with differential expression and up-regulation of TIPARP.

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Bevacizumab (Avastin) is an approved treatment option by the European Medicines Agency¹ for more than a quarter billion women in the European Union, and despite having its indication withdrawn by the Food and Drug Administration in 2011 is still utilized in clinical trials in the United States^{2,3}. We mined published microarray data⁴ from the PROMIX trial to understand in an unbiased fashion genes most transcriptionally perturbed by bevacizumab administration and how this interacted with a standard anthracycline and taxane chemotherapeutic regimen, epirubicin and docetaxel. We integrated these findings with analysis of a separate microarray dataset studying transcriptional changes globally in the tumors of patients with bladder cancer following treatment with a regimen including bevacizumab⁵. We report here the differential and increased expression of the TCDD inducible poly(ADP-ribose) polymerase, TIPARP, in the primary tumors of women treated with bevacizumab for breast cancer.

Keywords: breast cancer, TIPARP, bevacizumab, Avastin, transcription factors in breast cancer, systems biology of breast cancer, targeted therapeutics in breast cancer.

Breast cancer affects women worldwide with relatively high frequency⁶. Bevacizumab (Avastin), a monoclonal antibody targeted against the vascular endothelial growth factor A, VEGF-A, is utilized for the treatment of breast cancer worldwide⁷. Bevacizumab was formerly approved by the Food and Drug Administration in conjunction with paclitaxel for the treatment of metastatic breast cancer which had not previously been treated with chemotherapy for HER2-negative breast cancer; this indication was withdrawn on the basis that its full safety and efficacy had not been demonstrated². For the over quarter of a billion women in the European Union, bevacizumab remains approved by the European Medicines Agency for the treatment of metastatic breast cancer¹. To understand the transcriptional response to bevacizumab in an unbiased fashion at the transcriptome level, we mined published microarray data from PROMIX⁴, a phase II clinical trial measuring clinical responses to epirubicin, docetaxel and bevacizumab in women with breast cancer.

Methods

We used datasets GSE87455⁴ and GSE70691⁵ for this global differential gene expression analysis in conjunction with GEO2R. GSE87455 was generated using Illumina HumanHT-12 V4.0 expression beadchip technology with $n=122$ primary breast tumors at baseline, $n=83$ primary tumors after Cycle 2 of treatment, consisting of only chemotherapy (epirubicin and docetaxel) and $n=70$ following addition of bevacizumab to treatment regimen in patients with breast cancer; analysis was performed using platform GPL10558. GSE70691 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=22$ tumors of the bladder prior to treatment with methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) and bevacizumab, and $n=27$ tumors of the bladder post treatment with MVAC and bevacizumab; analysis was performed using platform GPL14951. 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 TIPARP gene expression was significantly different between primary breast tumors at baseline, following chemotherapy (epirubicin and docetaxel) only and following both chemotherapy and bevacizumab in humans with breast cancer using a one way analysis of variance (ANOVA) with Dunnett's multiple comparisons test. We used PRISM for all statistical analyses of differential gene expression in human breast cancer and murine endometrial cancer (Version 8.4.0)(455).

Results

We mined published microarray data^{4,5} to understand in an unbiased fashion and at the systems level how transcription globally was most significantly altered in primary tumors from humans following treatment with the monoclonal antibody bevacizumab.

Bevacizumab treatment results in differential expression and up-regulation of TIPARP in primary tumors of the breast in patients with breast cancer.

We analyzed data from the PROMIX trial⁴ wherein patients were treated for 6 cycles; each of the six cycles included treatment with epirubicin and docetaxel; bevacizumab was added to the treatment regimen only after cycle 2. Tumor tissues were sampled at baseline, following administration of 2 cycles of epirubicin and docetaxel (prior to administration of bevacizumab), and after the addition of bevacizumab, at surgery. We identified TIPARP as among the genes most differentially expressed in the primary tumors of human patients with breast cancer when comparing tumors at baseline, after 2 cycles of epirubicin and docetaxel, and at surgery, once bevacizumab had been administered for 4 cycles (Table 1). When sorting each of the genes expressed in the primary tumors of humans with breast cancer as compared to baseline, after 2 cycles of epirubicin and docetaxel, and after 4 cycles of bevacizumab,

epirubicin and docetaxel, for 6 cycles of total treatment, TIPARP ranked 200 out of 47323 total transcripts, equating to 99.6% differential expression. Differential expression of TIPARP in the primary tumors of women with breast cancer compared at baseline, after 2 cycles of epirubicin and docetaxel, and after 4 cycles of bevacizumab, epirubicin and docetaxel was statistically significant (Table 1; $p=6.87E-15$).

Next we evaluated the magnitude and direction of change in TIPARP gene expression in the primary tumors of patients treated with bevacizumab, after 2 cycles of epirubicin and docetaxel as well as once bevacizumab had been added to the treatment regimen for 4 cycles. We found that TIPARP significantly increased in expression following addition of bevacizumab to the treatment regimen (Figure 1; $p<0.0001$) but not following treatment with 2 cycles of epirubicin and docetaxel only (Figure 1; $p=0.0536$). We calculated a mean fold change of 0.85 ± 0.26 in TIPARP gene expression in primary tumors following 2 cycles of epirubicin and docetaxel chemotherapy, but a mean fold change of 1.48 ± 0.65 in TIPARP gene expression following addition of bevacizumab to the treatment regimen (Table 1).

A treatment regimen including bevacizumab results in differential expression of TIPARP in the tumors of patients with bladder cancer.

We turned to a second microarray dataset⁵ to understand more thoroughly tissue-specific transcriptional modulation of bevacizumab in cancer, comparing global gene expression between the tumors of patients with bladder cancer before and after treatment with methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) in conjunction with bevacizumab. We found that TIPARP was also among the genes most differentially expressed when comparing tumors of the bladder from patients before and after treatment with MVAC plus bevacizumab (Table 2). When sorting each of the genes expressed in tumors of the bladder based on significance of change in expression between untreated and MVAC-treated patients, TIPARP ranked 5017 out of 54675 total transcripts (Table 2), equating to 90.8% differential expression. Differential expression of TIPARP in MVAC plus bevacizumab-treated bladder tumors was statistically significant (Table 2; $p=5.75E-02$).

Thus, in at least two human cancers, we observed transcriptome-wide differential expression of TIPARP in the tumors of patients treated with bevacizumab or bevacizumab in conjunction with a treatment regimen. In patients with breast cancer, we observed a stunning induction of TIPARP in patients after bevacizumab was added to a treatment regimen including docetaxel and epirubicin, more significantly so than the marginal increase in TIPARP mRNA levels following 2 cycles of epirubicin and docetaxel, suggesting that differential expression and transcriptional induction of TIPARP in patient tumors is a direct result of treatment with bevacizumab and not other components of the treatment regimen.

Discussion

We provided evidence obtained through analysis of published microarray data^{4,5} that expression of TIPARP is among the genes most differentially expressed in the primary tumors of breast cancer patients treated with bevacizumab, docetaxel and epirubicin; further analysis revealed that TIPARP is significantly up-regulated in the tumors of patients with breast cancer following treatment with the monoclonal antibody bevacizumab in combination with a treatment regimen including docetaxel and epirubicin but not with docetaxel and epirubicin chemotherapy only.

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Rank	ID	p-value	F	FC	Gene	Gene name
200	ILMN_1765578	6.87E-15	3.68E+01	<u>B+E</u> : 0.85 ± 0.26 <u>B+E+D</u> : 1.48 ± 0.65	TIPARP	TCDD inducible poly(ADP-ribose) polymerase

Table 1: Differential expression of TIPARP in the primary breast tumors of women treated with bevacizumab.

The rank of differential expression, probe ID, *p*-value with respect to global differential expression, F-statistic, fold change in TIPARP transcription factor gene expression between primary tumors at baseline and primary tumors following administration of chemotherapy only (“E+D”: epirubicin and docetaxel), fold change in TIPARP transcription factor gene expression between primary tumors at baseline and primary tumors following addition of bevacizumab to epirubicin and docetaxel (“B+E+D”: bevacizumab, epirubicin and docetaxel) gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
5017	ILMN_1765578	5.75E-02	1.94	-4.37812	TIPARP

Table 2: Differential expression of TIPARP in patients treated with bevacizumab for bladder cancer.

The rank of differential expression, probe ID, *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change in TIPARP gene expression between tumors prior to treatment and tumors following administration of bevacizumab in conjunction with MVAC, gene and gene name are listed in this chart.

Figure 1: TIPARP is transcriptionally induced in the breast tumors of women more significantly following addition of bevacizumab to epirubicin and docetaxel treatment.

Expression of TIPARP at the messenger RNA level from the tumors of patients enrolled in the PROMIX trial is graphically depicted here in primary tumors at baseline (left), in primary tumors following administration of 2 cycles of chemotherapy only (epirubicin and docetaxel) (“Chemo”, middle), and in primary tumors at surgery, following addition of bevacizumab to treatment regimen (“Chemo + Bev”, right). with mean TIPARP mRNA level marked and the result of a statistical test evaluating significance of difference in TIPARP expression between primary tumors of the breast and metastases to the brain in patients with breast cancer, a p-value, listed above. ****: $p < 0.0001$. ns: not significant ($p = 0.0536$)