Bevacizumab treatment of patients with breast cancer is associated with differential expression and up-regulation of C10orf10.

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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Medicines Agency¹ for more than a quarter billion women in the European Union, and despite having its indication withdrawn by the Food and Drug Administration in 2011 is still utilized in clinical trials in the United States^{2,3}.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 findings from a separate microarray dataset generated using bevacizumab administration in an experimental model of endometrial cancer⁵. We report here the differential and increased expression of open reading frame 10 on chromosome 10, encoded by C10orf10 in the primary tumors of women treated with bevacizumab for breast cancer.

Keywords: breast cancer, C10orf10, 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cancer which had not previously been treated with chemotherapy for HER2-negative breast cancer; this indication was withdrawn on the basis that its full safety and efficacy had not been demonstrated².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 GSE18195⁵ 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. GSE18195 was generated using Affymetrix Human Genome U133 Plus 2.0 Array with $n=2$ primary tumors from a murine model of endometrial cancer (untreated control) and $n=2$ primary tumors following treatment with bevacizumab; analysis was performed using platform GPL570. 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 C10orf10 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 and from mice following treatment with the monoclonal antibody bevacizumab.

Bevacizumab treatment results in differential expression and up-regulation of C10orf10 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 open reading frame 10 on chromosome 10, encoded by C10orf10, 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, C10orf10 ranked 143 out of 47323 total transcripts, equating to 99.7% differential expression.

Differential expression of C10orf10 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; $p=3.19\text{E-}16$).

Next we evaluated the magnitude and direction of change in C10orf10 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 C10orf10 significantly increased in expression, but only after treatment with bevacizumab (Figure 1; $p<0.0001$ after treatment with trastuzumab, (Figure 1; $p=0.8496$) following treatment with 2 cycles of epirubicin and docetaxel only). We calculated a mean fold change of 2.05 ± 1.48 in C10orf10 gene expression in primary tumors following 2 cycles of epirubicin and docetaxel chemotherapy, but a mean fold change of 0.93 ± 0.54 in C10orf10 gene expression following addition of bevacizumab to the treatment regimen (Table 1).

Bevacizumab treatment results in differential expression of C10orf10 in the endometrial tumors of mice.

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 untreated tumors of murine endometrial tumors and the endometrial tumors of mice treated with bevacizumab. We found that C10orf10 was also among the genes most differentially expressed when comparing bevacizumab-treated murine endometrial tumors to untreated murine endometrial tumors (Table 2). When sorting each of the genes expressed in murine endometrial tumors based on significance of change in expression between untreated and bevacizumab-treated mice, C10orf10 ranked 6103 out of 54675 total transcripts (Table 2). Differential expression of C10orf10 in bevacizumab-treated murine endometrial tumors was not considered statistically significant (Table 2; $p=0.087488$) despite differential expression greater than 88.8% of the measured transcriptome.

Thus, differential expression of C10orf10 in association with bevacizumab administration is likely not simply a reflection of changes in tumor gene expression associated with clonal evolution, as its differential expression can be observed following administration in a controlled, experimental model.

Discussion

We provided evidence here through analysis of published microarray data^{4,5} that C10orf10 is among the genes most differentially expressed in the tumors of patients treated with bevacizumab for breast cancer and that this transcriptional modulation also occurs, to a less significant extent, in tumors isolated from a murine experimental model of endometrial cancer following administration of bevacizumab.

References

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Rank	ID	p-value	F	FC	Gene	Gene name
143	ILMN_1767556	3.19E-16	4.08E+01	<u>B+E</u> : 0.93 ± 0.54 <u>B+E+D</u> : 2.05 ± 1.48	C10orf10	chromosome 10 open reading frame 10

Table 1: Differential expression of C10orf10 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 C10orf10 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 C10orf10 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	Gene name
6103	209183_s_at	0.087488	2.1397936	-4.07565	C10orf10	chromosome 10 open reading frame 10

Table 2: Differential expression of C10orf10 in endometrial tumors of mice treated with bevacizumab.

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, gene and gene name are listed in this chart.

Figure 1: C10orf10 is transcriptionally induced in the breast tumors of women following addition of bevacizumab to epirubicin and docetaxel treatment but not following treatment with epirubicin and docetaxel only.

Expression of C10orf10 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 C10orf10 mRNA level marked and the result of a statistical test evaluating significance of difference in C10orf10 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; $p = 0.8496$.