Bevacizumab treatment of patients with breast cancer is associated with differential expression and up-regulation of the leukemia inhibitory factor receptor (LIFR).

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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Medicines Agency¹ for more than a quarter billion women in the European Union, and despite having its indication withdrawn by the Food and Drug Administration in 2011 is still utilized in clinical trials in the United States^{2,3}.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 report here the differential and increased expression of the leukemia inhibitory factor receptor, a gateway to a signaling pathway required for the pluripotency of embryonic stem cells^{5,6}, in the primary tumors of women treated with bevacizumab for breast cancer.

Keywords: breast cancer, LIFR, leukemia inhibitory factor receptor, 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 frequency⁷. Bevacizumab (Avastin), a monoclonal antibody targeted against the vascular endothelial growth factor A, VEGF-A, is utilized for the treatment of breast cancer worldwide⁸. 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cancer which had not previously been treated with chemotherapy for HER2-negative breast cancer; this indication was withdrawn on the basis that its full safety and efficacy had not been demonstrated².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 GSE18195⁹ 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, using a murine model of endometrial cancer, was generated using Affymetrix Human Genome U133 Plus 2.0 Array with $n=2$ primary tumors from mice untreated (control) and $n=2$ primary tumors from mice treated 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 LIFR transcription factor 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^{4,9} to understand in an unbiased fashion and at the systems level how transcription globally was most significantly altered in the primary tumors of human and mice following treatment with the monoclonal antibody bevacizumab.

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

We analyzed data from the PROMIX trial⁴ wherein patients with breast cancer 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 the leukemia inhibitory factor receptor, LIFR, 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,

LIFR ranked 70 out of 47323 total transcripts, equating to 99.9% differential expression. Differential expression of LIFR 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; $p=6.14\text{E-}19$).

Next we evaluated the magnitude and direction of change in LIFR 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 LIFR significantly increased in expression, but only after treatment with bevacizumab (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.96 ± 1.16 in LIFR gene expression in primary tumors following 2 cycles of epirubicin and docetaxel chemotherapy, but a mean fold change of 1.97 ± 1.17 in LIFR gene expression following addition of bevacizumab to the treatment regimen (Table 1).

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

We turned to a second microarray dataset⁹ to understand more thoroughly tissue-specific transcriptional modulation of bevacizumab in cancer, comparing global gene expression of tumors from a murine model of endometrial cancer, untreated, with that of endometrial tumors from this model following treatment with bevacizumab. We found that LIFR 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, LIFR ranked 3276 out of 54675 total transcripts (Table 2), equating to 94% differential expression. Differential expression of LIFR in bevacizumab-treated murine endometrial tumors was statistically significant (Table 2; $p=0.043835$).

Thus, differential expression of LIFR 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 both in primary tumors of the breast in humans with breast cancer and in primary tumors from mice treated with bevacizumab in a controlled, experimental model.

Discussion

Signal transduction mediated by the leukemia inhibitory factor is required for the pluripotency of murine embryonic stem cells in culture; provision of LIF can maintain pluripotency, which can be obviated by enforced expression of Nanog^{5,6}. Withdrawal of LIF from culture media results in loss of pluripotency and subsequent differentiation of ES cells in culture^{5,6}. LIF engagement of LIFR transduces signals through phosphorylation and activation of STAT3, which can directly activate transcription of Myc in the nucleus⁷⁻⁹. A study has described contribution of LIF towards metastasis and tumorigenesis in human breast cancer, acting through the protein kinase AKT-mTOR pathway¹⁰.

We provided evidence here that expression of the LIF receptor is up-regulated in the tumors of patients with breast cancer following treatment with the monoclonal antibody bevacizumab. We previously reported that four KLF transcription factors were transcriptionally induced in the primary tumors of patients treated with bevacizumab¹¹, two of which are important for induction and maintenance of pluripotency^{12,13}. While it has been argued that the dependency of ES cells on LIF signaling is different in humans from that in mouse, in that it is said to not be required for maintenance of pluripotency in human ES cells¹⁴⁻¹⁷, short-term expression of Nanog and KLF2 is sufficient to induce erasure of epigenetic marks genome-wide and reset human embryonic stem cells to ground state pluripotency¹⁴. Thus, bevacizumab, a therapeutic designed to treat and extinguish cancer, appears to paradoxically induce gene expression associated with a pluripotent stem cell state. Whether bevacizumab administration is associated with “stemness” and the generation of cancer stem cell clones remains to be evaluated, but a small molecule inhibitor of LIFR can reduce stemness in triple negative breast cancer cell lines and impair tumor progression in patient-derived xenografts¹⁸.

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Rank	ID	p-value	F	FC	Gene	Gene name
70	ILMN_1709094	6.14E-19	4.91E+01	E+D: 0.96 ± 1.16 B+E+D: 1.97 ± 1.17	LIFR	leukemia inhibitory factor receptor alpha

Table 1: Differential expression of the leukemia inhibitory factor receptor, LIFR, 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 LIFR 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 LIFR 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
3276	229185_at	0.043835	-2.713158	-3.46676	LIFR	leukemia inhibitory factor receptor alpha

Table 2: Differential expression of LIFR 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, fold change in LIFR gene expression between tumors at baseline and tumors following administration of bevacizumab, gene and gene name are listed in this chart.

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

Expression of LIFR at the messenger RNA level from the tumors of patients enrolled in the PROMIX trial is graphically depicted here at baseline (left), following administration of 2 cycles of chemotherapy only (epirubicin and docetaxel) (“Chemo”, middle), and at surgery, following addition of bevacizumab to treatment regimen (“Chemo + Bev”, right), with mean LIFR mRNA level marked and the result of a statistical test evaluating significance of difference in LIFR 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.