Changes in MMP11 expression in patients with breast cancer following treatment with bevacizumab.

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Bevacizumab, a monoclonal antibody targeting the vascular endothelial growth factor A (VEGF-A), is utilized in patients with metastatic breast cancer (1). We harnessed the power of unbiased, systems-level whole transcriptome comparative analyses, using published microarray data (2,3) to compare primary tumors of patients with breast cancer sampled at baseline and after treatment with bevacizumab (2), and the tumors of patients with colorectal cancer undergoing chemotherapy with or without bevacizumab (3). We report here that MMP11 was among the genes whose expression changed most significantly, transcriptome-wide, following bevacizumab treatment in patients with breast cancer. Modest but statistically significant differential expression of MMP11 was also observed in the tumors of colorectal cancer patients receiving adjuvant bevacizumab.

Keyword: bevacizumab, MMP11, systems biology of breast cancer, targeted therapeutics in breast cancer.

To facilitate an enhanced understanding of the transcriptional changes associated with bevacizumab treatment, we mined published microarray data (2) from the primary tumors of breast cancer patients enrolled in the PROMIX trial. We found that MMP11 was also among the genes whose expression changed most significantly in breast cancer patients after administration of bevacizumab but not after administration of epirubicin and docetaxel only. Modest differential expression of MMP11 was also observed in the primary tumors of colorectal cancer patients treated with FOLFIRI chemotherapy and adjuvant bevacizumab (3). We briefly describe these changes here.

Methods

We used datasets GSE87455 (2) and GSE72970 (3) for this global differential gene expression analysis in conjunction with GEO2R. GSE87455 (2) 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. GSE72970 (3) was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=60$ primary colorectal tumors in patients treated only with FOLFIRI chemotherapy and $n=22$ primary tumors of colorectal cancer patients treated with both FOLFIRI and 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 MMP11 gene expression was significantly 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 (Version 8.4.0)(455).

Results

MMP11 is among the genes whose expression changes most significantly, transcriptome-wide, in the primary tumors of patients with breast cancer following treatment with bevacizumab.

As bevacizumab is still indicated for use as first-line therapy in women in the European Union (4), and still utilized in clinical trials in the United States for women with breast cancer (5, 6) despite having its breast cancer indication withdrawn by the Food and Drug Administration in 2011 (7), we queried a microarray dataset generated using primary tumor samples obtained from women enrolled in the Phase II PROMIX trial to describe molecular changes in the tumors of women treated with bevacizumab. In this trial, women with localized HER2-negative primary breast cancer received six cycles of epirubicin and docetaxel for 6 cycles; bevacizumab was only added at cycle 3. Samples were taken from patients at baseline (before any chemotherapy), after cycle 2 (after patients had received only epirubicin and docetaxel), and after surgery (after patients had been treated with both chemotherapy and bevacizumab). Thus, we compared whole transcription in primary tumor samples obtained by core needle biopsy at three time points: baseline, after cycle 2 (chemotherapy only), and after bevacizumab had been added to the treatment regimen.

We found that MMP11 was also among the genes whose expression changed most significantly when comparing primary tumors from patients at baseline to that of patients following administration of bevacizumab (Table 3). When sorting each of these genes based on change in expression between primary tumors at baseline and primary tumors after administration of bevacizumab, MMP11 ranked 113 out of 47323 total transcripts. Differential expression of MMP11 in primary tumors following administration of bevacizumab was statistically significant (Table 1: $p=1.82E-16$).

Importantly, MMP11 was not differentially expressed when comparing primary tumors at baseline and following administration of 2 cycles of chemotherapy (epirubicin and docetaxel) (Table 2). When sorting each of the genes expressed in primary tumors following 2 cycles of chemotherapy (epirubicin and docetaxel) only as compared to primary tumors at baseline, MMP11 ranked 16043 out of 47323 total transcripts. This was not statistically significant (Table 2; $p=2.04E-01$). Thus, MMP11 expression is significantly transcriptionally modulated in primary tumors following addition of bevacizumab to treatment regimen, but not following administration of chemotherapy only. We concluded that modulation of MMP11 expression in primary tumors following treatment was likely associated with administration of bevacizumab rather than epirubicin or docetaxel.

Bevacizumab treatment, but not epirubicin and docetaxel chemotherapy, induces a significant decrease of MMP11 expression in primary tumors of patients with breast cancer.

We also obtained exact mRNA expression levels for MMP11 from the primary tumors of patients with breast cancer, at baseline prior to administration of chemotherapy, after 2 cycles of chemotherapy consisting of epirubicin and docetaxel only, and at surgery, following administration of bevacizumab in addition to chemotherapy, to determine magnitude and direction of change in MMP11 expression following administration of bevacizumab and determine if this change was statistically significant. MMP11 was expressed at significantly lower levels in the primary tumors of patients with breast cancer following addition of bevacizumab to treatment regimen, but not following administration of chemotherapy (epirubicin and docetaxel) only, when compared to baseline. We calculated a fold change in MMP11 expression of 0.229 ± 0.306 following bevacizumab administration but 0.777 ± 0.787 following administration of chemotherapy (epirubicin and docetaxel) only (Table 1 and Table 2, respectively). Change in MMP11 expression following administration of bevacizumab was statistically significant (Figure 1: $p<0.0001$), but change in MMP11 following administration of chemotherapy only was not statistically significant (Figure 1: $p=0.0974$).

Adjuvant bevacizumab is associated with modest differential expression of MMP11 in the primary tumors of patients with colorectal cancer.

We interrogated a separate published microarray dataset (2) that contained the results of an analysis comparing the transcriptomes of the primary tumors of patients with colorectal cancer treated only with FOLFIRI chemotherapy or with FOLFIRI chemotherapy and adjuvant bevacizumab for general validation and to evaluate tissue-specificity. We observed modest, but statistically significant differential expression of MMP11 in the tumors of colorectal cancer patients receiving adjuvant treatment with bevacizumab; log-fold induction data indicated that this change in expression was positive (increased in patients treated with adjuvant bevacizumab). When sorting each of the transcripts whose expression was measured globally by microarray based on significance of magnitude change, MMP11 ranked 8769 out of 54675

total transcripts, equating to 84.0% differential expression; this was statistically significant ($p=1.40E-02$).

Discussion

To understand the most significant transcriptional changes induced by bevacizumab treatment in the primary tumors of patients with breast cancer, we analyzed published microarray data (2) from the PROMIX trial, mining critical samples obtained from patients at baseline, after administration of chemotherapy (epirubicin and docetaxel) only, and at surgery after administration of bevacizumab. By integration of these analyses, we identified decreased expression of MMP11 in primary tumors of the breast following administration of bevacizumab. A separate dataset revealed differential expression of MMP11 in the primary tumors of patients with colorectal cancer receiving adjuvant bevacizumab (3). Future study can determine any clinical significance of these gene expression changes associated with bevacizumab administration in human breast cancer.

References

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Rank: 100
probe: 203878_s_at
p-value: 3.98E-04
t: -3.9214574
B: 0.0741
Fold change: 0.741 ± 0.155

Table 1: MMP11 is differentially expressed following administration of bevacizumab to patients with breast 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 when comparing primary tumors at baseline to primary tumors following administration of bevacizumab, fold change in MMP11 expression between primary tumors at baseline and primary tumors following administration of bevacizumab, gene and gene name are listed in this chart.

Rank: 113
probe: ILMN_1655915
p-value: 1.82E-16
t: -9.0322734
B: 26.857156
Fold change: 0.229 ± 0.306

Table 2: MMP11 is not differentially expressed following administration of chemotherapy only (epirubicin and docetaxel) to patients with breast 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 when comparing primary tumors at baseline to primary tumors following administration of chemotherapy only (epirubicin and docetaxel), fold change in MMP11 expression between primary tumors at baseline and primary tumors following administration of chemotherapy only (epirubicin and docetaxel), gene and gene name are listed in this table.

Figure 1: MMP11 is expressed at significantly lower levels in primary tumors of patients with breast cancer following administration of bevacizumab and chemotherapy but not following administration of chemotherapy only.

Expression of MMP11 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 MMP11 mRNA level marked and the result of a statistical test evaluating significance of difference in MMP11 expression between primary tumors of the breast and metastases to the brain in patients with breast cancer, a p-value, listed above. ns: not significant. *****: $p < 0.0001$. Because of the magnitude of difference in MMP11 expression between tumors at baseline and tumors following administration of bevacizumab, the scale (Y-axis) has been set to logarithmic.

Rank: 8769
probe: 235908_at
p-value: 1.40E-02
t: 2.5098613
B: -3.12692
Gene: MMP11
Gene name: matrix metalloproteinase

Table 3: Modest but statistically significant differential expression of MMP11 in the tumors of colorectal cancer patients receiving adjuvant 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 when comparing primary tumors of colorectal cancer patients receiving only FOLFIRI chemotherapy to that of colorectal cancer patients receiving FOLFIRI and bevacizumab, gene and gene name are listed in this chart.