Differential expression of the oxytocin receptor, OXTR, in cancers of the breast.

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Breast cancer affects women at relatively high frequency¹. We mined published microarray datasets^{2,3} to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We report here significant differential expression of the gene encoding the oxytocin receptor, OXTR, when comparing primary tumors of the breast to the tissue of origin, the normal breast. OXTR expression in primary tumors of the breast was significantly lower than in normal breast tissue, and relapse-free survival in breast cancer patients was significantly longer in patients with high tumor expression of OXTR than in patients with low tumor expression of OXTR. OXTR may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, OXTR, oxytocin receptor, systems biology of breast cancer, targeted therapeutics in breast cancer.

Invasive breast cancer is diagnosed in over a quarter of a million women in the United States each year¹ and in 2018, breast cancer was the leading cause of cancer death in women worldwide⁴. While patients with localized breast cancer are provided a 99% 5-year survival rate, patients with regional breast cancer, cancer that has spread to lymph nodes or nearby structures, are provided an 86% 5-year survival rate^{5,6}. Patients with metastasis to distant sites are provided a 27% 5-year survival rate^{5,6}. Understanding how primary tumors are most transcriptionally different from the tissue from which they originate, the breast, can facilitate development of novel diagnostic and therapeutics to promote early detection and enhanced treatment, and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data^{2,3} to understand at the transcriptome level and in an unbiased fashion genes most differentially expressed in primary tumors of the breast as compared to normal breast tissue. The oxytocin receptor emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE124648² and GSE109169³ and for this differential gene expression analysis of female breast cancer. GSE124648 was generated using Affymetrix Human Genome U133A Array technology with $n=10$ normal breast tissue and $n=126$ tumors of the breast and; analysis was performed using platform GPL96. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast and; analysis was performed using platform GPL5175. The tissues from this first dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). 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 OXTR expression was significantly between primary breast tumors and breast tissue using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of OXTR mRNA expression levels with relapse-free survival (RFS) in $n=3951$ breast cancer patients.

Results

We performed discovery of genes associated with breast cancer in females by mining two independently published microarray datasets^{2,3}.

OXTR is differentially expressed in primary tumors of the breast.

Comparison of 126 tumors of the breast to 10 normal breast tissues revealed that the gene encoding the oxytocin receptor, OXTR, was among the genes most differentially expressed in tumors of the breast in female breast cancer (Table 1). When sorting each of the genes expressed in primary breast tumors based on significance of difference as compared to normal breast tissue, OXTR ranked 4 out of 22283 total transcripts, equating to 99.98% differential expression (Table 1). Differential expression of OXTR in female breast cancer was statistically significant (Table 1; $p=4.87E-28$).

Analysis of a second microarray dataset³, here comparing 25 tumors of the breast to 25 normal breast tissues² again revealed significant differential expression of OXTR in tumors of the breast in female breast cancer (Table 2). When sorting each of the genes expressed in

tumors of the breast based on significance of difference as compared to normal breast tissue, OXTR ranked 387 out of 19076 total transcripts, equating to 98.0% differential expression (Table 2). Differential expression of OXTR in female breast cancer was again statistically significant (Table 2; $p=1.7E-11$).

OXTR is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for OXTR from the breast and from breast tumors to understand the magnitude and direction of OXTR expression change in human breast cancer. In both datasets, OXTR was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of OXTR in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). We calculated a mean fold change of 0.6921 ± 0.0680 when comparing primary tumors of the breast to normal breast tissues (Table 1).

OXTR expression associates with survival outcomes in breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate OXTR mRNA expression levels with survival outcomes in 3951 breast cancer patients. We found a statistically significant correlation between OXTR expression and relapse-free survival (RFS) in patients with breast cancer (Figure 2; log rank p -value: $1.4e-06$ for relapse-free survival, hazard ratio: 0.76 (0.68-0.85) (Fig. 2)). Low OXTR mRNA levels were a negative prognostic indicator in patients with breast cancer. Median RFS was 38 months for patients with low tumor expression of OXTR while median RFS was 60 months for patients with high tumor expression of OXTR (Table 3).

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and down-regulation of OXTR was among the most significant transcriptional features in primary tumors from patients with breast cancer. OXTR expression in primary tumors of the breast demonstrated a significant correlation with relapse-free survival in patients with breast cancer, with high expression of OXTR correlating with improved survival.

Discussion

The oxytocin hormone has wide-ranging functions in human behavior, including maternal behaviors⁸ and in trust⁹, as well as in physiologic functions such as in the induction of labor during child birth¹⁰. The receptor for the oxytocin receptor is expressed in the breast as a 3.6 kilobase transcript¹¹. Steroid hormones estrogen and progesterone both interact with the oxytocin hormone pathway. Progesterone can directly bind to the uterine oxytocin receptor and inhibit downstream calcium signaling and inositol triphosphate production, and in the mouse brain¹², the estrogen receptor alpha is required for estrogen-induced oxytocin up-regulation¹³.

Multiple studies, primarily in cancer cell lines, have documented expression of oxytocin and the oxytocin receptor in breast cancer. Expression of the oxytocin receptor has been described in endothelial cells purified from breast carcinomas (B-TEC), and oxytocin could induce cell proliferation and migration in B-TEC¹⁴. Gene expression analysis revealed that oxytocin stimulation induced expression of caveolin-1¹⁴. In contrast, a separate study demonstrated that in the triple negative breast cancer cell line MDA-MB-231, oxytocin stimulation decreased rather than increased cell proliferation¹⁵. Expression of the oxytocin receptor was found in multiple breast cancer cell lines, including MDA-MB-231, MCF7 and T47D¹⁵. Signaling through the oxytocin receptor by oxytocin was found to be mediated by cyclic

AMP (cAMP) and the protein kinase A pathway, and not through induction of inositol triphosphate and Ca²⁺ mobilization¹⁶. In MCF7 cells, oxytocin stimulation resulted in a decrease of estrogen receptor alpha (ER α) expression, but a concomitant increase in estradiol (E2)-induced transcriptional activity¹⁷. In MCF-7 and Hs 578T breast cancer cell lines, while progesterone stimulation decreased oxytocin receptor expression, E2 increased oxytocin receptor expression¹⁸. We identified one study describing expression of the oxytocin receptor in human breast cancer, detecting OXTR expression in 52 out of 57 human breast cancer tissues by immunohistochemistry, with 28 being diffusely positive and 24 being partially positive for OXTR expression¹⁹. In this study, however, in contrast to published decreased or increased breast cancer cell line growth in response to oxytocin stimulation, investigators found no change in cell proliferation of MCF-7, MFA-MB-231, MDA-MB-361 and MDA-MB-468 breast cancer cell lines after 7 days of oxytocin stimulation¹⁹. Thus, the oxytocin receptor, a receptor for a hormone with critical functions in human behavior and female physiology including lactation and parturition, is expressed in breast cancer cell lines; multiple studies have evaluated the effect of oxytocin stimulation on breast cancer cell proliferation with conflicting findings, and one study has documented positive expression of OXTR in human breast cancer tissues.

We report here that the oxytocin receptor, encoded by the OXTR gene is among the genes most differentially expressed transcriptome-wide in human breast cancer when comparing primary tumors of the breast to the tissue of origin, normal breast tissue. OXTR was expressed at significantly lower levels in primary breast tumors than in the breast, and high tumor expression of OXTR was associated with enhanced relapse-free survival in patients with breast cancer. The oxytocin receptor may be relevant to the initiation or progression of human breast cancer.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
4	206825_at	4.87E-28	-13.937402	52.95888	0.6921 ± 0.0680	OXTR	oxytocin receptor

Table 1: OXTR is differentially expressed in human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change of OXTR in primary breast tumors as compared to normal breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
387	2661992	1.7E-11	-8.60	15.999575	OXTR	oxytocin receptor

Table 2: OXTR is differentially expressed in human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Figure 1: OXTR is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of OXTR in normal breast tissue (left) and in primary tumors of the breast (right) is graphically depicted with the result of a statistical test evaluating significance of difference in OXTR expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Figure 2: OXTR expression correlates with relapse-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of relapse-free survival (RFS) for $n=3951$ total patients stratified into two groups, based on low or high expression of OXTR in patient primary tumors. The log rank p -value denoting statistical significance of difference in distant relapse-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on OXTR expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
38	60

Table 3: Median relapse-free survival is greater in breast cancer patients with high primary tumor expression of OXTR.

The relapse-free survival of $n=3951$ breast cancer patients based on stratification into low or high tumor expression of OXTR is listed in this chart.