

Olfactory receptors OR5B21 and OR6C76 are differentially expressed in humans with triple negative breast cancer

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Whole genome profiling data of 226 patients with triple negative breast cancer (1) was stratified based on survival. When comparing the transcriptomes of 87 patients that expired versus 123 patients that survived, two olfactory receptors were among the genes whose expression was most different. We previously reported that genes of the olfactory receptor superfamily were differentially expressed in metastases in the HER2+ Balb-NeuT mouse model of breast cancer (2). The data here indicate that differential expression of OR5B21 and OR6C76 associates with survival outcomes in humans with triple negative breast cancer.

Keywords: triple negative breast cancer, differential gene expression, OR5B21, OR6C76, systems oncology, targeted therapeutics in breast cancer

Introduction

Breast cancer affects over a quarter of a million women in the United States each year and is the second leading cause of cancer death behind lung cancer. In the United States alone, 42,000 women will die from this disease each year (3). Breast cancers can be classified based on the type of receptors they express, including the epidermal growth factor receptor, or HER2, the estrogen receptor, ER, and the progesterone receptor (4). Tumors that do not over-express HER2 and do not express the estrogen and progesterone receptor are known as triple negative breast cancers, or TNBC (4). Women diagnosed with TNBC suffer from a more challenging course of disease than other women diagnosed with breast cancer, including shorter time to relapse, shorter time to metastasis, more frequent metastasis, and shorter overall median survival (4). Standard chemotherapies target the rate at which cells divide, and not any intrinsic property of the cancer cell. Other chemotherapies such as tamoxifen target the hormone-receptor dependent properties of breast cancers, which TNBCs lack responsiveness to as they do not express the estrogen and progesterone receptors (4). More refined chemotherapies target such as the monoclonal antibody trastuzumab (5) target HER2+, which TNBC by definition does not over-express (4). There is a dire need for targeted chemotherapies for triple negative breast cancer.

I used a published dataset of microarray data from the tumors of 226 women diagnosed with triple negative breast cancer (1), or TNBC, to analyze on a systems-level what the most significant transcriptional differences are between the tumors of women that expire from this disease versus those that survived in this cohort. These analyses revealed that two olfactory receptors, olfactory receptor, family 6, subfamily C, member 21 (OR6C76) and olfactory receptor family 5, subfamily B, member 21 (OR5B21) were differentially expressed between women that survived and women that expired at time of analysis. We previously reported that several olfactory receptors are differentially expressed in a HER2+ model (2) and the findings

here support the assertion that olfactory receptors may be of significance to the etiology of this disease and/or for the development of novel targeted therapeutics.

Methods

Dataset GSE142102 (1) was used for this analysis using GEO2R. P-value adjustment was set to Benjamini and Hochberg; log transformation of data was set to “Auto-detect”, and the submitter supplied category of platform annotation was used. Statistical significance of differential expression was determined by GEO2R. Statistical significance to compare the mean value of RNA expression between dead and alive groups was performed using PRISM (PRISM 8.1.2) using a two-tailed, unpaired t-test with Welch’s correction. Graphs in 1A and 2A show standard error of the mean (SEM).

Results

We performed differential gene expression profiling using GEO2R on a dataset from a cohort of 266 African American women with triple negative breast cancers (1), whose tumors had been subject to microarray gene expression profiling. We stratified these patients based on survival at time of analysis, into two groups: dead and alive. We then asked which RNA message, system-wide, were the most different in terms of expression level between the tumors of the patients who had expired and those who had survived. We found that two olfactory receptors, OR6C76 and OR5B21 were the fifteenth and thirty-third most differentially expressed genes, respectively (Table 1), out of 53617 total transcripts detected, and their differential expression was statistically significant with respect to the entire tumor transcriptome (Table 1; OR6C76, $p = 0.00028098$; OR5B21, $p = 0.00070634$).

We next retrieved the exact RNA expression values for both OR6C76 and OR5B21 for each individual patient, and asked whether the mean expression value of each receptor between each group was statistically significant (versus whether the values of each receptor was significant with respect to change in expression relative to the rest of the transcriptome).

The expression of both OR6C76 and OR5B21 were significantly different when comparing patients who had expired versus those who were alive at time of analysis (Figure 1; OR6C76, $p = 0.0012$; Figure 2; OR5B21, $p = 0.0003$). While the expression of OR6C76 was higher in patients that had expired, the expression of OR5B21 was lower in patients that had expired.

Discussion

Breast cancer is a leading cause of cancer death for women in the United States (3). Triple negative breast cancer represents a more deadly form of the disease with fewer treatment options and a more aggressive course, and a relative lack of targeted treatments. There is a real need for targeted treatments for triple negative breast cancer (4). Systems-level analysis of the tumor transcriptome, by comparing normal tissue to tumor tissue, tumor tissue to metastatic tissue, or by comparing the tumor tissue of those with better and worse survival outcomes, can provide unbiased, global insights into the most significant transcriptional changes (6-12). These insights can be valuable for diagnostic, prognostic, and/or therapeutic purposes.

Here we performed global differential gene expression profiling on a cohort of 266 African American women with triple negative breast cancer (1), comparing the tumor transcriptomes of those who had expired versus those who had survived, in order to determine what the most significant transcriptional changes were in the primary tumors of women who had been stratified based on their survival outcomes. These analyses revealed that two olfactory receptors, OR6C76 and OR5B21 were the fifteenth and thirty-third most differentially expressed genes when comparing the transcriptomes of these patients based on survival.

We previously reported that several receptors of the olfactory receptor family were differentially expressed when comparing primary tumors to lung metastases in a HER2+ mouse model of metastatic breast cancer (2). These results indicate that across species, and across breast cancer types, expression of olfactory receptors is a most significant biological change in

cancers of the breast, and suggest that further understanding of these pathways in breast cancer and in normal mammary gland biology may be of relevance for therapeutic targeting of these receptors in triple negative breast cancer and perhaps in metastatic breast cancer of the HER2+ type.

References

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Table 1

Rank	Gene	NCBI ID	p-value	t	B	GenBank Accession
15	OR6C76	16752172	0.00028098	-3.6322353	-2.56	NM_001005183
33	OR5B21	16738628	0.00070634	3.3871081	-2.86	NM_001005218

Table 1: Genes encoding olfactory receptors are the 15th and 33rd most differentially expressed genes in a cohort of 266 women with triple negative breast cancer (TNBC): data from GEO2R whole transcriptome differential gene expression analysis.

Data provided here includes rank of differential expression relative to the transcriptome profiled, raw p-value for statistical significance of differential expression of the gene, log-odds that the gene is differentially expressed (B-statistic), and a moderated t statistic generated by comparison of the two groups (dead and alive).

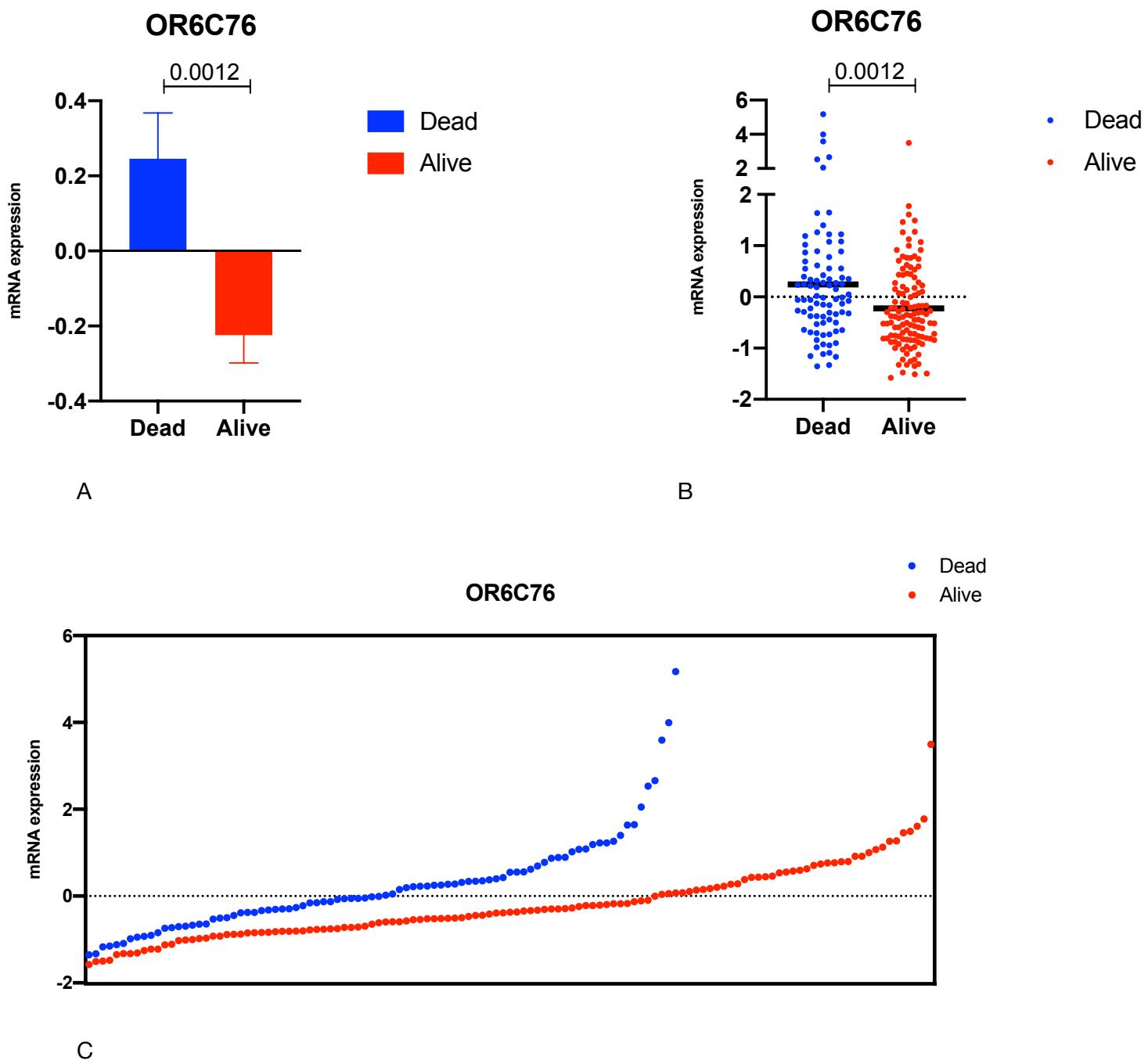
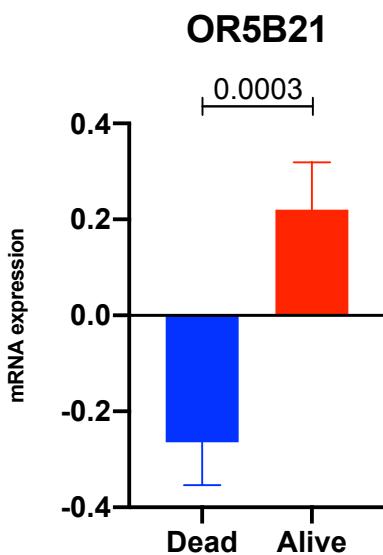


Figure 1: OR6C76 transcript is the fifteenth most differentially expressed gene in a cohort of 266 women with triple negative breast cancer (TNBC) when comparing survivors to the deceased.

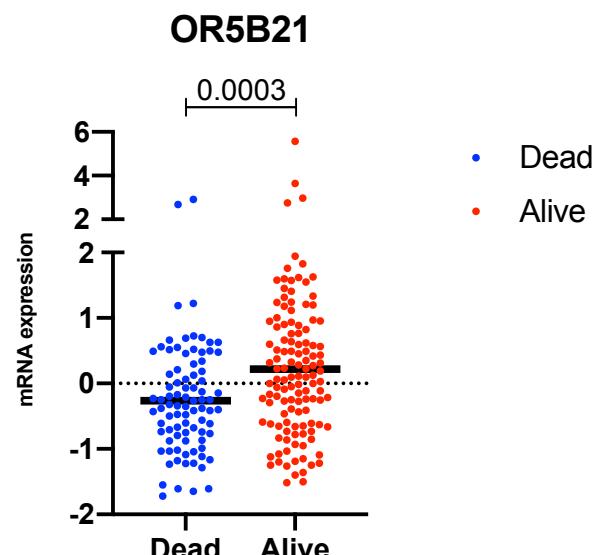
Figure 1A: The RNA expression value of OR6C76 in patients dead and alive, with the mean value designated by the horizontal black bar. Statistical significance as designated by the p-value above the graph, was calculated using an unpaired t-test with Welch's correction. Standard error of the mean is designated by the error bars.

Figure 1B: Mean expression of the OR6C76 transcript as in 1A but here each patient is represented by an individual dot. The black bar represents the mean value of the transcript, as calculated using an unpaired t-test with Welch's correction, and shown above the graph.

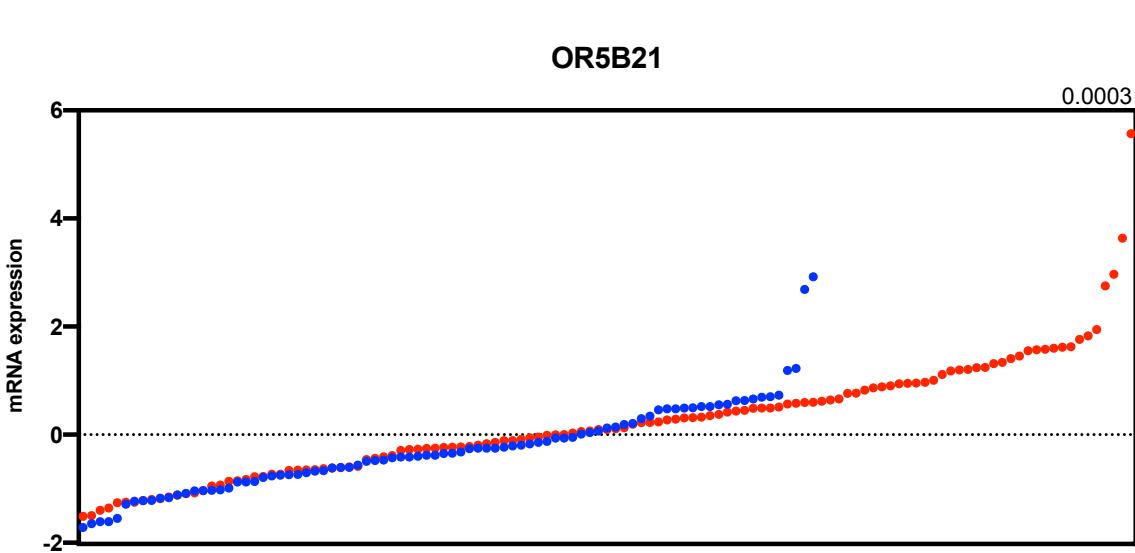
Figure 1C: The RNA expression value of OR6C76 in each patient is graphically represented by individual dots.



A



B



C

Figure 2: OR5B21 transcript is the thirty-third most differentially expressed gene in a cohort of 266 women with triple negative breast cancer (TNBC) when comparing survivors to the deceased.

Figure 2A: The RNA expression value of OR5B21 in patients dead and alive, with the mean value designated by the horizontal black bar. Statistical significance as designated by the p-value above the graph, was calculated using an unpaired t-test with Welch's correction. Standard error of the mean is designated by the error bars.

Figure 2B: Mean expression of the OR5B21 transcript as in 2A but here each patient is represented by an individual dot. The black bar represents the mean value of the transcript.

Figure 2C: The RNA expression value of OR5B21 in each patient is graphically represented by individual dots.