1 **The olfactory receptor gene OR56A4 is differentially expressed based on disease stage**
2 **in breast cancer.**

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7 To understand the transcriptional behavior that accompanies breast cancer progression, we
8 compared the global gene expression profiles of 100 tumors at stages I, II and III using two
9 independent published microarray datasets (1, 2). We found that the olfactory receptor, family
10 56, subfamily A, member 4 OR56A4 was among the genes whose expression was most
11 different when comparing stage I, stage II, and stage III primary tumors from patients with breast
12 cancer. OR56A4 expression was lower in stage III tumors as opposed to stage I tumors.
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26 **Keywords:** tumor transcriptome analysis, breast cancer, comparative differential gene
27 expression profiling, systems biology of breast cancer.
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Breast cancer was diagnosed in over 275,000 women in the United States last year (3). While 5-year survival rates for women diagnosed with stage I breast cancer is 99%, the 5-year survival rate for women with stage IV breast cancer is 27% (3). To understand the how the transcriptional behavior of primary tumors from women with breast cancer differs based on disease stage, we compared 100 primary tumor transcriptomes using two independently published microarray datasets (1, 2). Across both datasets, OR56A4 was among the genes most differentially expressed when comparing tumor transcriptomes based on disease stage.

Methods

We used datasets GSE109169 (1) and GSE86166 (2) for this differential gene expression analysis in conjunction with GEO2R. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array with n=25 tumors in total, with n=6 stage I, n=11 stage II, and n=7 stage III tumors from patients with early onset breast cancer. GSE86166 was generated using n=78 tumors in total with n=26 stage I, n=28 stage II, n=24 stage III tumors from patients with HER2+ and HER2- breast adenocarcinomas. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of 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 the significance of difference between OR56A4 transcription factor subunit expression levels in patient tumors based on stage, from stage I-III, using an ordinary one-way ANOVA with Dunnett's multiple comparisons test. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We compared tumor transcriptomes at stages I, II and III to understand, in an unbiased fashion, the transcriptional behavior of breast cancers based on disease progression level, using two independently published microarray datasets (1, 2).

OR56A4 is among the genes most differentially expressed in primary tumors when comparing tumor gene expression between disease stages I, II and III.

We identified the olfactory receptor, family 56, subfamily A, member 4 OR56A4 as among the genes most differentially expressed in the primary tumors of patients with breast cancer when comparing primary tumor transcriptomes based on disease stage, at stages I, II and III. When sorting all of the genes based on degree of change in expression between primary tumors of the breast based on disease stage, OR56A4 ranked 26 out of 19076 total transcripts. Differential expression of OR56A4 was statistically significant (Table 1; $p=0.0022128$).

By analyzing a separate microarray dataset, we again identified OR56A4 as among the genes most differentially expressed when comparing primary tumor transcriptomes based on disease stage, at stages I, II and III. When sorting all of the genes based on degree of change in expression between primary tumors of the breast based on disease stage, OR56A4 ranked 31 out of 60607 total transcripts. Differential expression of OR56A4 was again statistically significant (Table 1; $p=0.00011068$).

OR56A4 is expressed at significantly lower levels in stage III tumors when compared to stage I tumors in patients with breast cancer.

We extracted exact mRNA expression levels for OR56A4 from each patient tumor to directly compare OR56A4 gene expression between disease stages. OR56A4 was expressed at significantly lower levels in stage III tumors when compared to stage I tumors (Figure 1; $p=0.0033$). We calculated a 0.8458 ± 0.0710 fold change in OR56A4 expression in stage III tumors when compared to stage I tumors (Table 1).

In a separate dataset, we again found significantly decreased OR56A4 expression in stage III tumors when compared to stage I tumors from patients with breast cancer (Figure 2; $p=0.0008$). We calculated a 0.9103 ± 0.0591 fold change in OR56A4 expression in stage III tumors when compared to stage I tumors (Table 2).

Discussion

We sought to assess the transcriptional behavior of primary tumors of the breast based on disease stage in a systematic and unbiased fashion. Using two published microarray datasets (1, 2), we identified olfactory receptor OR56A4 as among the genes most differentially expressed when comparing tumors at stage I, stage II, and stage III.

OR56A4 has been demonstrated to act as a receptor for the ligands decanal and undecanal (4, 5). There is little information connecting these molecules to the development or progression of breast cancer, nor do we suggest there is such a relationship. Nevertheless, decanal is naturally produced by citrus and coriander and undecanal is found in citrus oils (6, 7).

One study surveyed olfactory receptor gene expression in a small sample size of primary breast tumors and suggested a role for olfactory receptor OR2B6 as a biomarker in breast cancer (8). A separate study linked OR2B6 expression with genes associated with proliferation (e.g., MKI67 and CCNB1), and found that OR2W3 expression was associated with the expression of genes involved in invasion (e.g., CTSV and MMP-11), suggesting that OR2W3 expression was an indicator of invasion or invasiveness (9). Another study documented increased expression of olfactory receptor OR2T6 in tumor tissues as compared to normal breast tissues, and found that OR2T6 expression was associated with poorer patient outcomes (10). *In vitro*, OR2T6 supported the proliferative and migratory properties of MCF-7 and MDA-MB-231 breast cancer cell lines (10). OR2T6 expression was associated with increased expression of mesenchymal markers (e.g., N-cadherin) and decreased expression of E-cadherin, and the authors concluded that OR2T6 may have a role in the epithelial-mesenchymal transition through activation of the MAP kinase pathway (10). A more comprehensive study analyzing the expression of all protein-coding OR genes found consistent with previously published studies that, OR2W3 and OR2T6 were upregulated in a panel of breast cancer cell lines (11). Both OR2W3 and OR2T6 were expressed at significantly higher levels in the tumors of patients with invasive breast cancer as compared to breast cancer cell lines (11).

Further studies can assess whether OR56A4 expression is a marker for disease progression in that its expression tends be lower in higher stage tumors, or whether there is a more fundamental relationship between OR56A4 expression and tumor progression in breast cancer. Moreover, while these data demonstrate OR56A4 expression is significantly lower in stage III tumors as compared to stage I tumors, demonstrating a negative relationship between tumor progression and OR56A4 mRNA expression, to conclude that OR56A4 expression

1 decreases with disease progression, as tumor stage increases, would require analysis of
2 lumpectomy samples from the same patient as their disease stage progressed.

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4 We found that the olfactory receptor OR56A4 was among the genes whose expression
5 was quantitatively mostly different between primary tumors of the breast at disease stages I, II
6 and III, expressed at significantly lower in stage III tumors as compared to stage I tumors.

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8 OR56A4 may be a biomarker for tumor progression in breast cancer.
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References

1. Chang, J.W., Kuo, W.H., Lin, C.M., Chen, W.L., Chan, S.H., Chiu, M.F., Chang, I.S., Jiang, S.S., Tsai, F.Y., Chen, C.H. and Huang, P.H., 2018. Wild-type p53 upregulates an early onset breast cancer-associated gene GAS7 to suppress metastasis via GAS7–CYFIP1-mediated signaling pathway. *Oncogene*, 37(30), pp.4137-4150.
2. Prabhakaran, S., Rizk, V.T., Ma, Z., Cheng, C.H., Berglund, A.E., Coppola, D., Khalil, F., Mulé, J.J. and Soliman, H.H., 2017. Evaluation of invasive breast cancer samples using a 12-chemokine gene expression score: correlation with clinical outcomes. *Breast Cancer Research*, 19(1), p.71.
3. ACS Cancer Facts & Figures 2019, <https://www.cancer.net/cancer-types/breast-cancer-metastatic/statistics>.
4. Adipietro, K.A., Mainland, J.D. and Matsunami, H., 2012. Functional evolution of mammalian odorant receptors. *PLoS genetics*, 8(7).
5. Mainland, J.D., Keller, A., Li, Y.R., Zhou, T., Trimmer, C., Snyder, L.L., Moberly, A.H., Adipietro, K.A., Liu, W.L.L., Zhuang, H. and Zhan, S., 2014. The missense of smell: functional variability in the human odorant receptor repertoire. *Nature neuroscience*, 17(1), p.114.
6. Nurzynska-Wierdak, R., 2013. Essential oil composition of the coriander (*Coriandrum sativum* L.) herb depending on the development stage. *Acta Agrobotanica*, 66(1).
7. Kohlpaintner, C., Schulte, M., Falbe, J., Lappe, P. and Weber, J., 2000. Aldehydes, aliphatic. *Ullmann's encyclopedia of industrial chemistry*.
8. Weber, L., Maßberg, D., Becker, C., Altmüller, J., Ubrig, B., Bonatz, G., Wölk, G., Philippou, S., Tannapfel, A., Hatt, H. and Gisselmann, G., 2018. Olfactory receptors as biomarkers in human breast carcinoma tissues. *Frontiers in oncology*, 8, p.33.
9. Masjedi, S., Zwiebel, L.J. and Giorgio, T.D., 2019. Characterization of olfactory receptor upregulation in invasive breast cancer. (2019): 3541-3541.
10. Li, M., Wang, X., Ma, R.R., Shi, D.B., Wang, Y.W., Li, X.M., He, J.Y., Wang, J. and Gao, P., 2019. The Olfactory Receptor Family 2, Subfamily T, Member 6 (OR2T6) Is Involved in Breast Cancer Progression via Initiating Epithelial-Mesenchymal Transition and MAPK/ERK Pathway. *Frontiers in oncology*, 9.
11. Masjedi, S., Zwiebel, L.J. and Giorgio, T.D., 2019. Olfactory receptor gene abundance in invasive breast carcinoma. *Scientific reports*, 9(1), pp.1-12.

Rank	ID	p-value	F	Fold change	Gene	Gene name
26	3360719 NM_001005179	0.0022128	8.003191	0.8458 ± 0.0710	OR56A4	olfactory receptor, family 56, subfamily A, member 4

Table 1: OR56A4 is differentially expressed in human breast cancers based on disease stage, when comparing the transcriptomes of stage I, stage II and stage III tumors.

The rank of differential expression, probe/transcript ID, the p-value of global differential expression, the F statistic, fold change, the gene and gene name are listed in this chart.

Rank	ID	p-value	F	Fold change	Gene	Gene name
31	merck- NM_001005179_at NM_001005179	0.00011068	1.03E+01	0.9103 ± 0.0591	OR56A4	olfactory receptor, family 56, subfamily A, member 4

Table 2: OR56A4 is differentially expressed in human breast cancers based on disease stage, when comparing the transcriptomes of stage I, stage II and stage III tumors.

The rank of differential expression, probe/transcript ID, the p-value of global differential expression, the F statistic, fold change, the gene and gene name are listed in this chart.

Figure 1: Expression of OR56A4 in stage III breast cancer patient tumors is significantly lower than in stage I patient tumors.

The mRNA expression level of OR56A4 is represented individually by patient with mean value marked. The result of a statistical test evaluating the significance of difference in expression of OR56A4 compared to stage I tumors, is listed above stage II and stage III tumor data. The p-value comparing expression between stage II and stage III tumors is listed above the indicated line.

Figure 2: Expression of OR56A4 in stage III breast cancer patient tumors is significantly lower than in stage I patient tumors.

The mRNA expression level of OR56A4 is represented individually by patient with mean value marked. The result of a statistical test evaluating the significance of difference in expression of OR56A4 compared to stage I tumors, is listed above stage II and stage III tumor data. The p-value comparing expression between stage II and stage III tumors is listed above the indicated line.