HER2+ murine breast cancers and lung metastases differentially express a series of olfactory and vomeronasal receptors

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Abstract

I previously reported that over 15% of the most differentially expressed genes in CD24+ CD73+ $\gamma\delta$ -T cells bearing a V γ 2 chain encode olfactory receptors. Here I present evidence that differential transcription of olfactory receptors can occur in breast cancers. Global comparative analysis of the transcriptomes of metastatic breast cancer in mice revealed that a series of Olfr (olfactory) genes were differentially expressed in tumors and in metastases. Lung metastases also expressed high levels of a non-coding RNA from the vomeronasal receptor family and two vomeronasal receptors. It is unknown whether any of these olfaction molecules play important roles in initiation or maintenance of tumors and/or metastases, or whether their expression can serve to improve diagnosis and/or prognosis. Differential expression of genes of the olfactory receptor family outside of the nervous system is not restricted to hematopoietic cells, and occurs in disease as well as in development.

Keywords: breast cancer, metastasis, olfactory receptors, vomeronasal receptors, non-coding RNA, Vmn1r42, Vmn2r7

Introduction

Breast cancer affects 268,8000 women and men every year in the United States, and 12% of women will develop invasive breast cancer sometime during their life (DeSantis et al., 2016). Over 42,000 of these women will succumb to the disease each year (DeSantis et al., 2016). Treatment consists of, depending on stage of disease: radiation, chemotherapy, and surgical removal of the tumor (lumpectomy/ mastectomy). Though the 5-year survival rate for women with localized disease is 99%, and 85% for women with disease that has spread to the lymph nodes, it is 27% for women with disease that has metastasized, or spread to distant organs such as the lungs (Mariotto et al., 2017). Novel therapeutic targets to halt or delay metastasis are of critical need.

Understanding how the breast cancer transcriptome is altered relative to healthy, non-cancerous tissue can enhance efforts towards the discovery of novel therapeutic targets; describing the transcriptional changes between primary tumor and metastasis are similarly crucial. Here I compared the global gene expression profiles of breast tumors to benign breast tissue, and breast tumors to lung metastases using a published dataset of a HER2+ BALB-NeuT mouse model of breast cancer (Hosseini et al., 2016). Salient features of this model include metastasis to the lungs and development of an independent tumor in each of 10 mammary glands (Iezzi et al., 2012).

I found that five of the genes most differentially expressed between metastases to the lung and primary tumor of the breast were receptors of the olfactory system. Eight additional genes of the olfactory system were among the most differentially transcribed when comparing primary tumors of the breast to healthy mammary tissue. Vomeronasal receptors were also significantly differentially expressed in primary tumors and in metastases relative to their counterparts (healthy mammary tissue and primary tumors), respectively. There is virtually no information or literature describing a relationship between metastatic behavior in breast cancer and the differential expression of olfactory and/or vomeronasal receptors.

Methods

Comparative Gene Expression Profiling

GEO (Gene Expression Omnibus) Dataset 68683 was utilized for this analysis (Hosseini et al., 2016). GEO Dataset 28598 was also used. The GEO2R web application, when provided with a dataset, identifies 250 transcripts whose expression is most differentially regulated in a given comparison between samples. Log transformation of data was set to “Auto-detect”, and the p-value, when adjusted, was set to “Benjamini-Yekutieli”. The “Submitter supplied” category of platform annotation was used to identify gene/RNA names.

Statistics

Statistical analyses comparing the mean RNA expression value of each transcript identified when comparing ([1] healthy mammary control breast tissue versus primary breast tumors, and [2] primary breast tumors versus lung metastases) were performed using GraphPad Prism Version 8.0. An unpaired t-test with Welch’s correction was performed for each gene, and gaussian distribution was assumed (parametric test). P-values for each comparison are denoted on each graph.

Results

Olfactory receptors are among the genes whose expression varies most significantly between primary breast tumor and metastases to the lung

The aim of this analysis was to describe the most significant differences between the transcriptional profiles of: (1) tumors of the breast (10 samples) and subsequent metastasis to (2) benign, control tissue (10 samples) and tumors of the breast (9 samples). GEO2R was utilized to analyze dataset GSE68683 in order to retrieve a rank-ordered list of the most significantly differentially expressed genes between either comparison (benign vs primary tumor: 183 genes, primary tumor vs metastasis: 199 genes). The genes most differentially expressed between primary tumors ($n=9$ samples from 6 mice) and metastases to the lung ($n=7$ samples from 3 mice) were analyzed first. An unpaired t test with Welch's correction was performed for each gene and the p -value is displayed for each differentially expressed transcript.

Of the 199 most significantly differentially expressed genes between primary tumors and metastases to the lung were five genes encoding olfactory receptors: Olfr297, Olfr476, Olfr516, Olfr862 and Olfr430. Olfr297, Olfr476 and Olfr516 displayed higher expression in metastases while Olfr862 and Olfr430 displayed lower expression in metastases when compared to primary tumors of the breast (Figure 1A and 1B). Two genes encoding vomeronasal receptors (V1rd6 and V1rd9) were also among the transcripts whose quantity varied most significantly between primary tumors and lung metastases (Figure 2A). In addition, I found that a non-coding RNA Vmn2r7 was differentially expressed, in higher quantities, in lung metastases when compared to primary breast tumors (Figure 2B).

Olfactory receptors are among the genes whose expression varies most significantly between healthy breast tissue and breast cancer in mice

Next, I compared the transcriptomes of primary tumors and benign, control tissue with the goal of determining the most differentially expressed genes between the two groups, again using microarray data generated from HER2 BALB-NeuT mice. I identified nearly 200 genes that displayed the highest variation in expression between primary breast tumors ($n=9$ samples from 6 mice), and benign tissue (healthy mammary cells; $n=10$ samples from 7 mice). An unpaired t test with Welch's correction was performed for each gene and the p -value is displayed for each differentially expressed transcript.

Eight of 183 transcripts I identified belonged to the family of olfactory receptors: Olfr906, Olfr386, Olfr78, Olfr488, Olfr601, Olfr285, Olfr452 and Olfr980 (Figure 3). Of these, all but one was expressed at significantly lower levels in primary breast tumors than in healthy mammary tissue; Olfr980 was expressed at significantly higher levels in primary breast tumors than in control mammary tissue. Again, two receptors that encode vomeronasal receptors, V1rc8 and V1ra1 were among the most differentially expressed transcripts when comparing primary breast tumors to healthy mammary tissue (Figure 4).

The gene expression of an olfactory receptor is influenced by high-fat diet

Finally, I used GEO Profiles to ask whether any information could be mined regarding the potential mechanisms by which the olfactory or vomeronasal systems might influence the biology of cancer and metastasis.

Brief Report

In an independent dataset that examined the role of TGF-beta signaling in obesity and diabetes (Yadav et al., 2011), I found that the expression of Vmn1r42 (V1ra6) was significantly influenced by the consumption of a high-fat diet in diet-induced obese (DIO) mice, but not by TGF-beta signaling blockade through Smad3 deletion or through the use of a TGF-beta antibody (Figure 5). It has been demonstrated across multiple populations that a high-fat diet is a major contributor to breast carcinogenesis in humans (Lee et al., 1991, Pierce et al., 2007, Willett et al., 1987). Thus, it can be concluded that a major risk factor for the development of breast cancer in humans, a high-fat diet, has a significant effect on the expression of a vomeronasal receptor in mice *in vivo*.

Conclusion

Analysis of a microarray dataset using the HER2 Balb-NeuT mouse model of breast cancer revealed that five of the most differentially expressed genes when comparing metastases to the lung and primary tumors of the breast were genes encoding olfactory receptors. Similarly, nine of the most differentially expressed genes when comparing breast tumors to benign breast tissue were olfactory receptors.

A previous study identified human olfactory receptor OR2B6 as a potential biomarker in breast cancers. Transcript for OR2B6 was identified in 73% of all breast cancer cell lines tested, as well 80% of tested breast tumor tissue (Weber et al., 2018). However, mechanistic studies investigating the role of OR2B6 in tumor initiation or propagation were not performed.

I also determined that the expression of a vomeronasal receptor (V1ra6) that I previously identified as differentially regulated in $\gamma\delta$ -T cells is significantly decreased by high-fat diet consumption in diet-induced obese mice. Whether the human homologue(s) of V1ra6 play any role in breast tumorigenesis linked to a high-fat diet is unknown but merits further investigation.

The differential expression of any gene in disease could have implications in its diagnosis or prognosis. It is unknown whether any of the olfactory receptors named here whose expression is significantly different between tumor and breast or tumor and metastasis could be used as a marker in aiding breast cancer diagnosis/patient stratification, or in conjunction with outcomes data be used to inform patient prognosis. Similarly, whether these genes play a role in the progression or maintenance of breast tumorigenesis or metastases is unknown. Differential expression of genes of the olfactory receptor family is not restricted to hematopoietic cells, and occurs in disease as well as in development.

References

1. Conti, L., Ruiu, R., Barutello, G., Macagno, M., Bandini, S., Cavallo, F. and Lanzardo, S., 2014. Microenvironment, oncoantigens, and antitumor vaccination: lessons learned from BALB-neuT mice. *BioMed research international*, 2014.
2. DeSantis, C.E., Fedewa, S.A., Goding Sauer, A., Kramer, J.L., Smith, R.A. and Jemal, A., 2016. Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. *CA: A Cancer Journal for Clinicians*, 66(1), pp.31-42.
3. Hosseini, H., Obradović, M.M., Hoffmann, M., Harper, K.L., Sosa, M.S., Werner-Klein, M., Nanduri, L.K., Werno, C., Ehrl, C., Maneck, M. and Patwary, N., 2016. Early dissemination seeds metastasis in breast cancer. *Nature*, 540(7634), p.552.

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4. Iezzi, M., Calogero, R.A., Spadaro, M., Musiani, P., Forni, G. and Cavallo, F., 2012. BALB-neuT Female Mice as a Dynamic Model of Mammary Cancer. *Translational Animal Models in Drug Discovery and Development*, p.139.
5. Lee, H.P., Lee, J., Gourley, L., Duffy, S.W., Day, N.E. and Estève, J., 1991. Dietary effects on breast-cancer risk in Singapore. *The Lancet*, 337(8751), pp.1197-1200.
6. Mariotto, A.B., Etzioni, R., Hurlbert, M., Penberthy, L. and Mayer, M., 2017. Estimation of the number of women living with metastatic breast cancer in the United States. *Cancer Epidemiology and Prevention Biomarkers*, 26(6), pp.809-815.
7. Pierce, J.P., Natarajan, L., Caan, B.J., Parker, B.A., Greenberg, E.R., Flatt, S.W., Rock, C.L., Kealey, S., Al-Delaimy, W.K., Bardwell, W.A. and Carlson, R.W., 2007. Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the Women's Healthy Eating and Living (WHEL) randomized trial. *Jama*, 298(3), pp. 289-298.
7. 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.
8. Willett, W.C., Stampfer, M.J., Colditz, G.A., Rosner, B.A., Hennekens, C.H. and Speizer, F.E., 1987. Dietary fat and the risk of breast cancer. *New England Journal of Medicine*, 316(1), pp. 22-28.
9. Yadav, H., Quijano, C., Kamaraju, A.K., Gavrilova, O., Malek, R., Chen, W., Zervas, P., Zhigang, D., Wright, E.C., Stuelten, C. and Sun, P., 2011. Protection from obesity and diabetes by blockade of TGF- β /Smad3 signaling. *Cell metabolism*, 14(1), pp.67-79.

Figure 1: Differential expression of olfactory receptors in lung metastases ($n=7$ samples from 3 mice) and primary tumors of the breast ($n=9$ samples from 6 mice). Each dot represents one tissue sample, and the + mark denotes the mean mRNA expression value.

A: Genes that were expressed at significantly higher levels in lung metastases.
B: Genes that were expressed at significantly lower levels in lung metastases.

Figure 2: A: Differential expression of vomeronasal receptors in lung metastases ($n=7$ samples from 3 mice) and primary tumors of the breast ($n=9$ samples from 6 mice). Each dot represents one tissue sample, and the + mark denotes the mean mRNA expression value.

B: Differential expression of a vomeronasal receptor non-coding RNA in lung metastases; same conditions as 2A. The exact RNA expression value for each individual sample is shown.

Figure 3: Differential expression of olfactory receptors in healthy mammary tissue ($n=10$ samples from 6 mice) and primary tumors of the breast ($n=9$ samples from 6 mice). Each dot represents one tissue sample, and the + mark denotes the mean mRNA expression value.

Figure 4: Differential expression of vomeronasal receptors in healthy mammary tissue ($n=10$ samples from 6 mice) and primary tumors of the breast ($n=9$ samples from 6 mice). Each dot represents one tissue sample, and the + mark denotes the mean mRNA expression value.

Figure 5: mRNA for vomeronasal receptor Vmn1r42 (V1ra6) is significantly reduced in the diet-induced obesity (DIO) mouse model. $n=2$ for each condition; the standard deviation is denoted by upper and lower bars.