

ZFX expression is connected with improved survival in patients with breast cancer

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Abstract

We analyzed published tumor transcriptome data in conjunction with linked survival data to identify genes linked with breast cancer survival outcomes (1, 2). When comparing tumor transcriptomes based on 24-month survival, we discovered that the zinc-finger protein X-linked ZFX (3, 4) was among the most differentially expressed genes in both original and metastatic tumor tissues. ZFX expression was considerably enhanced in metastatic tumors of patients who survived more than 24 months, indicating that enhanced ZFX expression offers a survival advantage for patients with stage IV metastatic breast cancer.

Keywords: metastatic breast cancer, targeted therapeutics in breast cancer, transcriptome

INTRODUCTION

Patients with metastatic breast cancer have an unacceptably low 5-year overall survival rate (OS) of 27% (5). To aid in the development of therapeutic targets and a better understanding of the fundamental transcriptional nature of metastatic breast cancer, we mined previously published microarray data from original and metastatic tumors of patients with breast cancer, together with linked survival outcome data (1, 2). By comparing the transcriptomes of metastatic breast cancer tissues from patients, we discovered that ZFX was one of the genes whose expression substantially varied between those who survived more than or less than 24 months. When comparing the transcriptomes of initial breast cancers, analysis of a different dataset indicated that ZFX expression was likewise related with disease-free survival at 24 months. These findings contradict existing evidence demonstrating that elevated ZFX expression is related with lower survival outcomes in individuals with breast cancer.

METHOD

We conducted this comparative differential gene expression study of the breast cancer transcriptome using the datasets GSE48391 (1) and GSE124647 (2) in combination with GEO2R. GSE48391 (1) was created using Affymetrix Human Genome U133 Plus 2.0 Array technology with n=70 primary breast tumors from women with more than 24 months of disease-free survival and n=11 primary breast tumors from women with fewer than 24 months of disease-free life. GSE124647 (2) was derived from biopsies of patients with stage IV metastatic breast cancer with a n=70 for metastatic tumor tissues from women who survived longer than 24 months and a n=70 for metastatic tumor tissues from women who survived less than 24 months. The Benjamin and Hochberg technique were used to rank differential expression, however raw p-values were utilized to determine the statistical significance of global differential expression. The log-transformation of data was discovered automatically, and the NCBI-generated platform annotation category was utilized. A statistical test was used to determine the significance of the difference in ZFX mRNA expression levels between primary tumors of women who survived less than 24 months and primary tumors of women who survived more than 24 months, as well as between metastatic tissues of women who survived less than 24 months and metastatic tissues of women who survived more than 24 months. Statistical significance was defined as p-values less than 0.05. We conducted all statistical analyses using PRISM (Version 8.4.0).

RESULT

We mined existing microarray data in order to conduct a comparative transcriptome study of breast cancer tumors and identify genes associated with improved survival outcomes. ZFX expression is significantly higher in primary breast cancer patients with a disease-free survival of longer than 24 months relative to those with a disease-free survival of less than 24 months. When comparing transcriptomes depending on disease-free survival at 24 months, we discovered ZFX as one of the most differently expressed genes in patient tumors. When each microarray-measured transcript was ranked according to its change in expression between tumors from patients with less than 24 months of disease-free survival and tumors from patients with more than 24 months of disease-free survival, a transcript encoded by the ZFX gene was ranked 229 out of 54674 transcripts. ZFX expression was significantly different in primary breast cancers from patients with a disease-free survival of more than 24 months. ZFX expression is significantly increased in the metastatic tumour cells of patients with breast cancer who have an overall survival of more than 24 months compared to those who have an overall survival of less than 24 months. Additionally, we investigated a second microarray dataset that included transcriptome data from metastatic tissues from several organ locations in patients with primary IV metastatic breast cancer. Again, when we compared tumour transcriptomes depending on overall survival at 24 months, we found ZFX as one of the most differently expressed genes in tumor metastases (Table 2). When each microarray-measured transcript was ranked according to its change in expression between metastatic tumors from patients with a median overall survival of more than 24 months and tumor metastases from patients with a median overall survival of less than 24 months, a transcript encoded by the ZFX gene was ranked 137th out of 22283 transcripts. ZFX expression was significantly different in primary breast cancers from patients with a disease-free survival of more than 24 months. ZFX is expressed at considerably higher levels in the primary tumors of patients with breast cancer who have a disease-free survival of longer than 24 months compared to those with disease-free survival of less than 24 months. We collected precise quantities of ZFX mRNA expression from each patient tumor and used a statistical test to assess if the difference in expression between survival groups was statistically significant. ZFX expression was considerably higher in the primary tumors of breast cancer patients with a disease-free survival more than or equal to 24 months than in those with a disease-free survival less than or equal to 24 months, and this difference was statistically significant. Between surviving groups, we determined a fold change of 1.05 between them. ZFX expression is considerably higher in metastatic tumor tissues from patients with breast cancer who have an overall survival of more than 24 months than in original tumors from patients with breast cancer who have an overall survival of less than 24 months. Additionally, we determined the precise ZFX mRNA expression levels in each metastatic tumor tissue and used a statistical test to assess if the difference in expression between survival groups was statistically significant. ZFX expression was considerably higher in metastatic tumor tissues of stage IV metastatic breast cancer patients with an overall survival of longer than 24 months than in those with an overall survival of less than 24 months, and this difference was statistically significant. Between surviving groups, we computed a fold change of 1.19. Thus, when comparing patients' survival outcomes at 24 months, we found ZFX as a differentially expressed gene in human breast cancer, both in original and metastatic tumors. ZFX expression was considerably increased in original tumors from patients with breast cancer who had a disease-free survival of more than 24 months. ZFX expression was considerably increased in metastatic tumors from patients with an overall survival of more than 24 months in patients with stage IV metastatic breast cancer.

DISCUSSION

The ZFX gene is an X-chromosome gene that contains 13 zinc fingers and is identical to the ZFY gene on the Y chromosome, save for 10 amino acids out of 393 total amino acids (3, 4). ZFX is required for embryonic stem cell (ESC) self-renewal (6). ZFX-deficient embryonic stem cells are incapable of self-

renewal but retain their differentiation potential. ZFX is also required for the function and survival of hematopoietic stem cells (HSCs) (6). ZFX deficit in Tie2-Cre mice resulted in the loss of adult HSC, but residual knock-out HSC retained normal homing, egress, and erythromyeloid progenitor activity (6). However, complete knockouts were uncommon, indicating that there is significant selection against ZFX loss in the HSC (6). Primitive hematopoiesis was normal in the yolk sac of Tie2-Cre Zfx-deficient mice, demonstrating that ZFX is essential for adult hematopoiesis but not for primitive hematopoiesis (6). After injection with pIpC, mice reconstituted with Mx-Cre Zfx-deficient bone marrow lose their ability to produce B cells, T cells, granulocytes, dendritic cells, and the majority of NK cells, and there is a significant increase in Annexin-V positive apoptotic HSC, demonstrating that ZFX is required for self-renewal, maintenance, and survival of HSC in vivo (6). ZFX is a novel chemical that has the capacity to regulate both embryonic and hematopoietic stem cell features (6, 7). Throughout the hematological system, ZFX has been implicated in BCR-induced B-cell proliferation and survival (8). ZFX was also shown to be an inhibitor of acute T-lymphoblastic leukemia and myeloid leukemia differentiation (9), and was revealed to be required for imatinib resistance and proliferation of chronic myeloid leukemia cells (10). In non-hematopoietic malignancies, ZFX has been implicated in tumor development and cell proliferation in a number of solid tumors. Gastric cancer, gliomas and glioblastomas, squamous cell carcinomas of the tongue, pancreatic cancer, renal carcinoma, gallbladder cancer, and colorectal cancer are only a few examples (11-19). Three independent investigations concluded that elevated ZFX expression is pro-tumorigenic and/or promotes tumor development in breast cancer (20-22). According to one of these investigations, suppressing ZFX resulted in a reduction in breast cancer cell growth (20). Another investigation discovered a connection between increased ZFX expression and metastatic progression (21). A third research discovered that the long non-coding RNA was critical for triple negative breast cancer cells through ZFX suppression by microRNA 218. (22). On the other hand, our data demonstrate that increased ZFX expression is associated with improved survival outcomes, that ZFX is one of the most differentially expressed genes in those surviving stage IV metastatic cancer for more than 24 months, and that ZFX is expressed at higher levels in the primary and metastatic tumors of human breast cancer patients with a median survival of more than 24 months. According to one research, ZFX has a role in carcinogenesis via activating the Hedgehog pathway (23). ZFX has been shown to target histone genes (24) and the MHC-Class I HLA-11 promoter (25). ZFX expression differential expression in breast cancer should be verified at the protein level and in bigger and distinct cohorts of breast cancer patients. As we discovered that patients with better survival outcomes expressed greater levels of ZFX, the impact of imposed ZFX expression on tumor cell proliferation and viability in vitro and on tumor growth, metastasis, and survival in xenograft breast cancer models in vivo should be studied. In this investigation, we discovered that ZFX was one of the genes with the greatest quantitative difference in expression between tumors from patients with localized and metastatic breast cancer based on 24-month survival outcomes. ZFX expression was considerably increased in the main tumors of patients with breast cancer who had a disease-free survival of more than 24 months. ZFX expression was considerably increased in metastatic tumor tissues from stage IV metastatic breast cancer patients with an overall survival of more than 24 months. Our data together show that ZFX expression is related with improved survival outcomes in individuals with breast cancer, particularly those with metastatic breast cancer at stage IV. Modulating ZFX expression in breast cancer may be a logical treatment approach.

References

- [1] Waks AG, Winer EP. Breast cancer treatment: a review. JAMA. 2019;321:288–300.
- [2] Burstein MD, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin. Cancer Res. 2015;21:1688–1698.

- [3] Aggarwal C, et al. SWOG S1400D (NCT02c965378), a phase III study of the fibroblast growth factor receptor inhibitor AZD4547 in previously treated patients with fibroblast growth factor pathway-activated stage IV squamous cell lung cancer (lung-MAP substudy) *J. Thorac. Oncol.* 2019;14:1847–1852.
- [4] Edelman MJ, et al. SWOG S1400C (NCT02154490)-a phase III study of paalbociclib for previously treated cell cycle gene alteration-positive patients with stage IV squamous cell lung cancer (lung-MAP substudy) *J. Thorac. Oncol.* 2019;14:1853–1859.
- [5] Herbst RS, et al. Lung master protocol (lung-MAP)-a biomarker-driven protocol for accelerating development of therapies for squamous cell lung cancer: SWOG S1400. *Clin. Cancer Res.* 2015;21:1514–1524.
- [6] Rodon J, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. *Nat. Med.* 2019;25:751–758.
- [7] O'Shaughnessy J, et al. Phase III study of iniparib plus gemcitabine and carboplatin versus gemcitabine and carboplatin in patients with metastatic triple-negative breast cancer. *J. Clin. Oncol.* 2014;32:3840–3847.
- [8] Fröhlich, B. and Plate, J. 2000. The cubic mouse: a new device for three-dimensional input. In *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems*
- [9] Stott JRR. Orientation and disorientation in aviation. *Extreme Physiology & Medicine.* 2013; 2: 2.doi: 10.1186/2046-7648-2-2?optIn=true.
- [10] Newman RL, Rupert AH. The magnitude of the spatial disorientation problem in transport airplanes. *Aerospace Med Hum Perf.* 2020; 91(2): 65-70.doi: 10.3357/AMHP.5442.2020.
- [11] Gillingham KK. A primer of vestibular function, spatial disorientation, and motion sickness. *Aeromedical Reviews.* 1966; 4: 1-80.
- [12] Upton K, Modi A, Patel K, et al. Epigenomic profiling of neuroblastoma cell lines. *Sci Data.* 2020;7(1):116. Published 2020 Apr 14. doi:10.1038/s41597-020-0458-y
- [13] Rokita JL, Rathi KS, Cardenas MF, et al. Genomic Profiling of Childhood Tumor Patient-Derived Xenograft Models to Enable Rational Clinical Trial Design. *Cell Rep.* 2019;29(6):1675-1689.e9. doi:10.1016/j.celrep.2019.09.071
- [14] Shiff NJ, Oen K, Rabbani R, Lix LM. Validation of administrative case ascertainment algorithms for chronic childhood arthritis in Manitoba, Canada. *Rheumatol Int.* 2017;37(9):1575-1584. doi:10.1007/s00296-017-3734-1
- [15] Stuart S, Hickey A, Vitorio R, et al. Eye-tracker algorithms to detect saccades during static and dynamic tasks: a structured review. *Physiol Meas.* 2019;40(2):02TR01. Published 2019 Feb 26. doi:10.1088/1361-6579/ab02ab
- [16] Mahajan V, Venugopal VK, Murugavel M, Mahajan H. The Algorithmic Audit: Working with Vendors to Validate Radiology-AI Algorithms-How We Do It. *Acad Radiol.* 2020;27(1):132-135. doi:10.1016/j.acra.2019.09.009
- [17] Buxton, E. K., Vohra, S., Guo, Y., Fogleman, A., & Patel, R. (2019). Pediatric population health analysis of southern and central Illinois region: A cross sectional retrospective study using association rule mining and multiple logistic regression. *Computer methods and programs in biomedicine*, 178, 145–153.
- [18] Campbell E. Random Compiler for Fast Hamiltonian Simulation. *Phys Rev Lett.* 2019;123(7):070503. doi:10.1103/PhysRevLett.123.070503
- [19] Devkota S, Aschwanden P, Kunen A, Legendre M, Isaacs KE. CcNav: Understanding Compiler Optimizations in Binary Code. *IEEE Trans Vis Comput Graph.* 2021;27(2):667-677. doi:10.1109/TVCG.2020.3030357

- [20] Yang X, He H. An advanced compiler designed for a VLIW DSP for sensors-based systems. *Sensors (Basel)*. 2012;12(4):4466-4478. doi:10.3390/s120404466
- [21] Minkovich K, Srinivasa N, Cruz-Albrecht JM, Cho Y, Nogin A. Programming time-multiplexed reconfigurable hardware using a scalable neuromorphic compiler. *IEEE Trans Neural Netw Learn Syst*. 2012;23(6):889-901. doi:10.1109/TNNLS.2012.2191795
- [22] Patel, Rushabh, and Yanhui Guo. "Graph Based Link Prediction between Human Phenotypes and Genes." *ArXiv:2105.11989 [Cs]*, May 2021. arXiv.org, <http://arxiv.org/abs/2105.11989>.
- [23] Lunter G. HMMoC--a compiler for hidden Markov models. *Bioinformatics*. 2007;23(18):2485-2487. doi:10.1093/bioinformatics/btm350