GSR expression associates with survival in triple negative breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

We mined published microarray data (1) to understand the most significant gene expression differences in the tumors of triple negative breast cancer patients based on survival following treatment: dead or alive. We observed significant transcriptome-wide differential expression of glutathione reductase, encoded by GSR when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, GSR expression was correlated with overall survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. GSR may be of relevance as a biomarker or as a molecule of interest in understanding the etiology or progression of triple negative breast cancer.

Keywords: triple negative breast cancer, TNBC, systems biology of breast cancer, targeted therapeutics in breast cancer, GSR, glutathione reductase.

Triple negative breast cancer is defined by lack of expression of the receptors for the hormones progesterone and estrogen (PR negative and ER negative), as well as for the human epidermal growth factor receptor 2 (HER2 negative) (2, 3). Though not completely identical, triple negative breast cancer shares significant overlap with the basal or basal-like molecular subtype of human breast cancer (3). TNBC is more frequently diagnosed in women of African descent (black women) for reasons not understood, and their disease course is similarly more aggressive than in white women with triple negative breast cancer (2, 3). To understand the transcriptional biology of triple negative breast cancer in an unbiased fashion and at the systems level, we mined published microarray data paired with patient survival data (1), performing comparative transcriptome analysis based on survival at time of data collection: dead or alive. We present here a finding from the results of this analysis.

Methods

We used dataset GSE142102 (1) for this differential gene expression analysis in conjunction with GEO2R. GSE142102 was generated using Affymetrix Human Gene 2.1 ST Array technology; in this analysis, we used data from $n=87$ tumors from TNBC patients that were dead at time of analysis and $n=123$ tumors from TNBC patients that were alive at time of analysis. 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 GSR expression was significantly different between the primary tumors of TNBC patients that were dead or alive using a two-tailed t -test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool (4) for correlation of GSR mRNA expression levels with overall survival (OS) in $n=431$ patients with basal subtype breast cancer.

Results

We performed comparative transcriptome analysis using published microarray data (1) to understand in an unbiased fashion the most significant gene expression differences in the primary tumors of patients with triple negative breast cancer based on survival outcomes.

GSR is differentially expressed in triple negative breast cancer and negatively correlates with survival.

When comparing primary tumors of 87 live patients with triple negative breast cancer with that of 123 dead TNBC patients we identified glutathione reductase, *GSR*, as among the most significant transcriptional differences based on survival in human triple negative breast cancer. When ranking each of the transcripts whose expression was measured by microarray based on significance of difference in expression between the tumors of patients dead and alive, *GSR* ranked 11 out of 53617 total transcripts, equivalent to 99.98% differential expression (Chart 1). *GSR* differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00022708$).

GSR is expressed at significantly lower levels in the breast tumors of TNBC patients when comparing based on survival.

We obtained exact mRNA expression levels for *GSR* from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of *GSR* expression change based on survival outcomes. *GSR* was expressed at lower levels in tumors of TNBC patients alive as compared to that of

1 TNBC patients dead (Figure 1). Decreased expression of GSR in primary breast tumors was statistically
2 significant (Figure 1: $p=0.0003$). GSR was expressed at 0.31 ± 1.06 arbitrary units (AU) in the primary
3 tumors of TNBC patients dead, while it was expressed at -0.20 ± 0.92 AU in the primary tumors of TNBC
patients alive.

4 GSR expression correlates with overall survival in basal subtype human breast cancer.

5 We referenced a bioinformatics tool containing Kaplan-Meier survival data of human breast cancer
6 patients to understand more thoroughly whether a relationship existed between GSR primary tumor
7 expression and patient survival in human breast cancer. We found that GSR expression was correlated
8 with overall survival in patients with basal or basal-like human breast cancer, a breast cancer molecular
9 subtype which shares significant overlap with triple negative breast cancer, in the lower quartile (Figure 2;
10 log rank p -value: 0.026 for overall survival, hazard ratio: 0.63 (0.42-0.95) (Fig. 2)). GSR mRNA levels
were a positive prognostic indicator in basal subtype breast cancer patients. Median OS was 44.4 months
for basal subtype patients with low tumor expression of GSR as compared to 89.04 months for basal
subtype patients with high tumor expression of GSR.

11 Thus, we found through unbiased comparative transcriptome analysis that GSR was among the
12 genes whose expression was most significantly different in the primary tumors of triple negative breast
13 cancer patients alive when compared to that of TNBC patients dead, that GSR was expressed at
14 significantly lower levels in the primary tumors of TNBC patients alive as compared to TNBC patients
dead, and that GSR expression was positively correlated with overall survival in the basal or basal-like
molecular subtype.

15 **Discussion**

16 It is not understood why women of African descent (black women) are diagnosed with triple
17 negative breast cancer more frequently than white women, and why this disease is more aggressive in
18 black women. GSR has been described as important for disparate processes that share requirements for
19 the redox cycle including oxidative burst during phagocytic behavior by neutrophils for host defense
20 against bacteria (5) as well as resistance to temozolomide in glioblastoma cells (6). GSR may be of
21 relevance as a biomarker in identifying patients who are more likely to survive, perhaps as part of a panel
22 of genes used for prognostic stratification, and the molecule itself may be important to the biology of triple
negative breast cancer etiology or more likely its progression. Blind systems-level analyses, like this, can
help shed light on the fundamental molecular characteristics of a disease whose racial propensities demand
further explanation.

References

1. Purrington, K.S., Knight III, J., Dyson, G., Ali-Fehmi, R., Schwartz, A.G., Boerner, J.L. and Bandyopadhyay, S., 2020. CLCA2 expression is associated with survival among African American women with triple negative breast cancer. *PloS one*, 15(4), p.e0231712.
2. Foulkes, W.D., Smith, I.E. and Reis-Filho, J.S., 2010. Triple-negative breast cancer. *New England journal of medicine*, 363(20), pp.1938-1948.
3. Dietze, E.C., Sistrunk, C., Miranda-Carboni, G., O'regan, R. and Seewaldt, V.L., 2015. Triple-negative breast cancer in African-American women: disparities versus biology. *Nature Reviews Cancer*, 15(4), pp.248-254.
4. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*, 123(3), pp.725-731.
5. Yan, J., Meng, X., Wancket, L.M., Lintner, K., Nelin, L.D., Chen, B., Francis, K.P., Smith, C.V., Rogers, L.K. and Liu, Y., 2012. Glutathione reductase facilitates host defense by sustaining phagocytic oxidative burst and promoting the development of neutrophil extracellular traps. *The Journal of Immunology*, 188(5), pp.2316-2327.
6. Zhu, Z., Du, S., Du, Y., Ren, J., Ying, G. and Yan, Z., 2018. Glutathione reductase mediates drug resistance in glioblastoma cells by regulating redox homeostasis. *Journal of neurochemistry*, 144(1), pp.93-104.

Rank: 11
Probe ID: 17076063
p-value: 0.00022708
t: 3.6868101
B: -2.5
Gene: GSR
Gene name: glutathione reductase

Chart 1: GSR is differentially expressed at the transcriptome-level when comparing the primary tumors of TNBC patients dead and alive.

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: GSR is expressed at significantly lower levels in the primary tumors of TNBC patients alive as compared to the primary tumors of TNBC patients dead.

The mRNA expression level of GSR in the primary tumors of TNBC patients dead (left) and in primary tumors of TNBC patients alive (right) is graphically depicted with the result of a statistical test evaluating significance of difference in GSR expression between the primary tumors of TNBC patients dead and alive, a *p*-value, listed here: *p*=0.0003.

Figure 2: GSR expression correlates with overall survival in basal subtype human breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=431$ breast cancer patients of basal subtype, based on low or high expression of GSR in patient primary tumors, in the lower quartile. The log rank p-value denoting statistical significance of difference in recurrence-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 GSR 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 GSR expression: 44.4 months
High GSR expression: 89.04 months

Chart 2: Median overall survival is superior in basal subtype breast cancer patients with high primary tumor expression of GSR.

The median OS (overall survival) of basal subtype breast cancer patients, with low primary tumor expression of GSR and high primary tumor expression of GSR is listed in this chart, in the lower quartile.