AES 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 amino-terminal enhancer of split, encoded by AES when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, AES expression was significantly correlated with overall survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer, in patients whose tumors contained a mutation in p53. AES 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, AES, amino-terminal enhancer of split.

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 AES 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 AES mRNA expression levels with overall survival (OS) in $n=64$ patients with p53 mutant 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.

AES 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 amino-terminal enhancer of split, *AES*, 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, AES ranked 241 out of 53617 total transcripts, equivalent to 99.6% differential expression (Chart 1). AES differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00585068$).

AES 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 AES from breast tumors of TNBC patients dead

and alive to understand the magnitude and direction of AES expression change based on survival outcomes. AES was expressed at lower levels in tumors of TNBC patients alive as compared to that of TNBC patients dead (Figure 1). Decreased expression of AES in primary breast tumors was statistically significant (Figure 1: $p=0.0039$). AES was expressed at 0.21 ± 0.87 arbitrary units (AU) in the primary tumors of TNBC patients alive, while it was expressed at -0.17 ± 1.03 AU in the primary tumors of TNBC patients dead.

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

We referenced a bioinformatics tool containing Kaplan-Meier survival data of human breast cancer patients to understand more thoroughly whether a relationship existed between AES primary tumor expression and patient survival in human breast cancer. We found that AES expression was correlated with overall survival in patients with basal or basal-like human breast cancer, a breast cancer molecular subtype which shares significant overlap with triple negative breast cancer, in the upper quartile, in patients whose tumors contained a mutation in the tumor suppressor p53 (Figure 2; log rank p -value: 0.052 for overall survival, hazard ratio: 2.54 (0.96-6.7) (Fig. 2)); this correlation approached statistical significance. AES mRNA levels were a negative prognostic indicator in basal subtype breast cancer patients. Median OS was 108 months for basal subtype p53 mutant patients with low tumor expression of AES as compared to 24.07 months for basal subtype p53 mutant patients with high tumor expression of AES.

Thus, we found through unbiased comparative transcriptome analysis that AES was among the genes whose expression was most significantly different in the primary tumors of triple negative breast cancer patients alive when compared to that of TNBC patients dead, that AES was expressed at significantly lower levels in the primary tumors of TNBC patients alive as compared to TNBC patients dead, and that AES expression was negatively correlated with overall survival in the basal or basal-like molecular subtype, in patients whose tumors contained a mutation in p53.

Discussion

It is not understood why women of African descent (black women) are diagnosed with triple negative breast cancer more frequently than white women, and why this disease is more aggressive in black women. AES is an inhibitor of both NF- κ B and androgen receptor pathway gene expression, and has been suggested to function as a tumor suppressor in the prostate. AES may be of relevance as a biomarker in identifying patients who are more likely to survive, perhaps as part of a panel 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. Ono, T., Sonta, S.I., Tetsuka, T., Uranishi, H., Imai, H., Takahashi, N., Asamitsu, K. and Okamoto, T., 2000. Inhibition of nuclear factor- κ B-mediated transcription by association with the amino-terminal enhancer of split, a Groucho-related protein lacking WD40 repeats. *Journal of Biological Chemistry*, 275(6), pp.4383-4390.
6. Yu, X., Li, P., Roeder, R.G. and Wang, Z., 2001. Inhibition of androgen receptor-mediated transcription by amino-terminal enhancer of split. *Molecular and cellular biology*, 21(14), pp.4614-4625.
7. Okada, Y., Sonoshita, M., Kakizaki, F., Aoyama, N., Itatani, Y., Uegaki, M., Sakamoto, H., Kobayashi, T., Inoue, T., Kamba, T. and Suzuki, A., 2017. Amino-terminal enhancer of split gene AES encodes a tumor and metastasis suppressor of prostate cancer. *Cancer science*, 108(4), pp.744-752.

Rank: 241
Probe ID: 16867047
p-value: 0.00585068
t: 2.756035
B: -3.51
Gene: AES
Gene name: amino-terminal enhancer of split

Chart 1: AES 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: AES 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 AES 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 AES expression between the primary tumors of TNBC patients dead and alive, a *p*-value, listed here: *p*=0.0039.

Figure 2: AES expression correlates with overall survival in basal subtype human breast cancer, in patients with mutant p53.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=64$ breast cancer patients of basal subtype, based on low or high expression of AES in patient primary tumors. 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 AES 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 AES expression: 108 months
High AES expression: 24.07 months

Chart 2: Median overall survival is superior in basal subtype p53 mutant breast cancer patients with low primary tumor expression of AES.

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