Differential expression of histone cluster 1, H2ag in triple negative breast cancer.

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Women diagnosed with triple negative breast cancer can benefit neither from endocrine therapy nor from HER2-targeted therapies¹. We mined published microarray datasets^{2,3} to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We report here significant differential expression of the gene encoding histone cluster 1, H2ag, HIST1H2AG, when comparing the tumor cells of patients with triple negative breast cancer to normal mammary ductal cells². HIST1H2AG was also differentially expressed in bulk tumor in human breast cancer³. HIST1H2AG mRNA was present at significantly increased quantities in TNBC tumor cells relative to normal mammary ductal cells. Analysis of human survival data revealed that expression of HIST1H2AG in primary tumors of the breast was correlated with post-progression survival in patients with basal-like type cancer, while within triple negative breast cancer, primary tumor expression of HIST1H2AG was correlated with recurrence-free survival in patients with basal-like 1 and luminal androgen receptor subtype disease. HIST1H2AG may be of relevance to initiation, maintenance or progression of triple negative breast cancers.

Keywords: breast cancer, HIST1H2AG, histone cluster 1, H2ag, systems biology of breast cancer, targeted therapeutics in breast cancer.

Patients with hormone receptor positive disease can benefit from endocrine therapy⁴: aromatase inhibition such as that provided by exemestane or letrozole, or estrogen receptor antagonism such as that provided by tamoxifen or fulvestrant. Patients whose tumors overexpress the human epidermal growth factor 2 (HER2) can be treated with HER2-targeted therapies: HER2 inhibitors, immunoglobulin⁵- or small molecule-based⁶. As triple negative breast cancer (TNBC) is defined by its lack of HER2 expression as well as by its lack of expression of the hormone receptor estrogen, patients diagnosed with triple negative breast cancer will not benefit from either of these treatment strategies.

Understanding the transcriptional composition of triple negative breast cancers in humans can facilitate development of novel therapeutics and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data^{2,3} to understand at the transcriptome level and in an unbiased fashion genes most differentially expressed in the tumor cells of patients with triple negative breast cancer.

Methods

We utilized datasets GSE38959² and GSE109169³ for this differential gene expression analysis of female breast cancer in conjunction with GEO2R. GSE38959 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=13$ samples of normal mammary gland ductal cells and $n=30$ samples of tumor cells from patients with triple negative breast cancer; analysis was performed using platform GPL4133. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast; analysis was performed using platform GPL5175. The tissues from this dataset are paired tissues (25 tumors matching 25 normal breast tissues from 25 patients). 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 HIST1H2AG expression was significantly different between primary breast tumors and breast tissue using a two-tailed t-test.

For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool⁷ for correlation of HIST1H2AG mRNA expression levels with post-progression survival (PPS) in $n=128$ patients with luminal A subtype cancer, $n=148$ patients with luminal B subtype cancer, and $n=77$ patients with basal-like subtype cancer, $n=92$ patients with HER2+ cancer. In patients with triple negative breast cancer⁷, we analyzed recurrence-free survival (RFS) for $n=251$ patients with basal-like 1 subtype TNBC, $n=101$ patients with basal-like 2 subtype TNBC, $n=300$ patients with immunomodulatory subtype TNBC, $n=253$ patients with luminal androgen receptor subtype TNBC, $n=211$ patients with mesenchymal subtype TNBC, and $n=81$ patients with mesenchymal stem-like subtype TNBC.

Results

We performed discovery of genes associated with triple negative breast cancer in humans by mining two independently published microarray datasets^{2,3}.

HIST1H2AG is differentially expressed in primary tumors and tumor cells of the breast.

Comparison of the tumor cells of patients with triple negative breast cancer to normal mammary ductal cells² revealed histone cluster 1, H2ag, encoded by HIST1H2AG, as among the genes whose expression was most significantly different in human TNBC as compared to the normal breast (Chart 1). When sorting each of the genes expressed in the tumor cells of patients with triple negative breast cancer based on significance of difference as compared to normal mammary ductal cells, HIST1H2AG ranked 4 out of 45015 total transcripts, equating to 99.99% differential expression (Chart 1). Differential expression of HIST1H2AG in the tumor cells of patients with triple negative breast cancer was statistically

significant (Chart 1; $p=8.48E-16$).

Analysis of a second microarray dataset³, here comparing 25 normal breast tissues to 25 tumors of the breast revealed that HIST1H2AG was also among the genes most differentially expressed in human breast cancer, here in the tumors of women diagnosed with early-onset breast cancer (Chart 2). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, HIST1H2AG ranked 2331 out of 19076 total transcripts, equating to 87.8% differential expression (Chart 2). Differential expression of HIST1H2AG in female breast cancer was statistically significant (Chart 2; $p=1.98E-06$).

These data indicated that differential expression of HIST1H2AG was not an artifact of a single microarray dataset, was not an artifact of FACS isolation, and suggested that perturbed expression of HIST1H2AG was likely a transcriptional feature of triple negative breast cancers and early-onset human breast cancers.

HIST1H2AG is expressed at significantly higher levels in triple negative tumor cells as compared to normal cells of the breast.

We obtained exact mRNA expression levels for HIST1H2AG - from normal mammary ductal cells of the breast and from tumor cells of patients with triple negative breast cancer - to understand the magnitude and direction of HIST1H2AG expression change in TNBC. HIST1H2AG mRNA transcript was present at higher levels in triple negative tumor cells of the breast as compared to normal mammary ductal cells (Figure 1). Increased expression of HIST1H2AG in primary tumor-isolated triple negative breast cancer cells was statistically significant (Figure 1: $p<0.0001$). HIST1H2AG was expressed at 180.8 ± 211.7 arbitrary units (A.U.) in normal mammary ductal cells, while it was expressed at 1262.0 ± 516.8 A.U. in tumor cells of patients with triple negative breast cancer. We calculated a mean fold change of 7.0 when comparing normal mammary ductal cells to triple negative breast cancer tumor cells.

HIST1H2AG expression correlates with survival outcomes in basal-like human breast cancer.

We performed Kaplan-Meier survival analysis to evaluate potential relationships between primary tumor HIST1H2AG mRNA expression levels and survival outcomes in patients with breast cancer, when analyzing outcomes based on the molecular subtype of the patient (PAM50 classification): luminal A, luminal B, HER2+ and basal-like. We observed a correlation between HIST1H2AG expression and post-progression survival (PPS) in patients with basal-like subtype breast cancer which approached statistical significance (Figure 2; log rank p -value: 0.066 for post-progression survival, hazard ratio: 0.59 (0.33-1.04) (Fig. 2)).

HIST1H2AG mRNA levels were a positive prognostic indicator in basal-like breast cancer patients. Median PPS was 17.16 months for basal-like patients with low tumor expression of HIST1H2AG while median PPS was 33.7 months for basal-like patients with high tumor expression of HIST1H2AG (Chart 3).

HIST1H2AG primary tumor expression was not correlated with post-progression survival in luminal A subtype (Figure 2; log rank p -value: 0.94 for PPS, hazard ratio: 1.02 (0.63-1.65) (Fig. 2), luminal B subtype (Figure 2; log rank p -value: 0.33 for PPS, hazard ratio: 1.22 (0.82-1.81) (Fig. 2)), or in HER2+ breast cancer (Figure 2; log rank p -value: 0.13 for PPS, hazard ratio: 0.68 (0.42-1.12) (Fig. 2)).

Influence of primary tumor HIST1H2AG expression on survival outcomes based on TNBC molecular subtype.

We also performed Kaplan-Meier survival analysis to examine relationships between HIST1H2AG mRNA expression levels with survival strictly in patients with triple negative breast cancer, analyzing

outcomes based on the TNBC molecular subtype of the patient: basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor. We observed a correlation between HIST1H2AG expression and recurrence-free survival (RFS) in TNBC patients with basal-like 1 subtype disease which trended towards statistical significance (Figure 3; log rank p -value: 0.076 for recurrence-free survival, hazard ratio: 1.48 (0.96-2.29) (Fig. 3)). HIST1H2AG mRNA levels were a negative prognostic indicator in patients with basal-like 1 TNBC. Median RFS was 25.2 months for basal-like 1 TNBC patients with low tumor expression of HIST1H2AG while median RFS was 19 months for basal-like 1 TNBC patients with high tumor expression of HIST1H2AG (Chart 4).

We also observed a statistically significant correlation between HIST1H2AG expression and recurrence-free survival (RFS) in TNBC patients with luminal androgen receptor subtype disease (Figure 3; log rank p -value: 0.034 for recurrence-free survival, hazard ratio: 1.51 (1.03-2.21) (Fig. 3)). HIST1H2AG mRNA levels were a negative prognostic indicator in patients with basal-like 1 TNBC. Median RFS was 27 months for luminal androgen receptor TNBC patients with low tumor expression of HIST1H2AG while median RFS was 15 months for luminal androgen receptor TNBC patients with high tumor expression of HIST1H2AG (Chart 4).

HIST1H2AG primary tumor expression was not correlated with recurrence-free survival in basal-like 2 subtype (Figure 3; log rank p -value: 0.55 for RFS, hazard ratio: 0.83 (0.45-1.53) (Fig. 3), immunomodulatory subtype (Figure 3; log rank p -value: 0.58 for RFS, hazard ratio: 0.88 (0.56-1.39) (Fig. 3)), in the mesenchymal subtype (Figure 3; log rank p -value: 0.18 for RFS, hazard ratio: 1.31 (0.88-1.95) (Fig. 3)), or in the mesenchymal stem-like subtype (Figure 3; log rank p -value: 0.81 for RFS, hazard ratio: 0.92 (0.45-1.86) (Fig. 3)).

Discussion

We performed comparative transcriptome analysis using two independently published microarray datasets^{2,3} to define and catalog transcriptional differences in human triple negative breast cancer. We provided evidence here that histone cluster 1, H2ag, encoded by the HIST1H2AG gene, is a defining transcriptional feature of triple negative breast cancer. HIST1H2AG was also differentially expressed in the bulk tumors of patients with early-onset breast cancer. Expression of HIST1H2AG was correlated with post-progression survival in patients with basal-like subtype breast cancer, and within triple negative breast cancer patients, primary tumor expression of HIST1H2AG was correlated with recurrence-free survival in patients with basal-like 1 and luminal androgen receptor subtype disease. Histone cluster 1, H2ag and the molecular processes to which it pertains to may be relevant to the initiation or progression of triple negative breast cancer in humans.

References

1. 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.
2. Komatsu, M., Yoshimaru, T., Matsuo, T., Kiyotani, K., Miyoshi, Y., Tanahashi, T., Rokutan, K., Yamaguchi, R., Saito, A., Imoto, S. and Miyano, S., 2013. Molecular features of triple negative breast cancer cells by genome-wide gene expression profiling analysis. *International journal of oncology*, 42(2), pp.478-506.
3. 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.
4. Spring, L.M., Gupta, A., Reynolds, K.L., Gadd, M.A., Ellisen, L.W., Isakoff, S.J., Moy, B. and Bardia, A., 2016. Neoadjuvant endocrine therapy for estrogen receptor–positive breast cancer: a systematic review and meta-analysis. *JAMA oncology*, 2(11), pp.1477-1486.
5. Slamon, D., Eiermann, W., Robert, N., Pienkowski, T., Martin, M., Press, M., Mackey, J., Glaspy, J., Chan, A., Pawlicki, M. and Pinter, T., 2011. Adjuvant trastuzumab in HER2-positive breast cancer. *New England journal of medicine*, 365(14), pp.1273-1283.
6. Park, J.W., Liu, M.C., Yee, D., Yau, C., van't Veer, L.J., Symmans, W.F., Paoloni, M., Perlmutter, J., Hylton, N.M., Hogarth, M. and DeMichele, A., 2016. Adaptive randomization of neratinib in early breast cancer. *New England Journal of Medicine*, 375(1), pp.11-22.
7. 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.

Rank: 4
Probe ID: 25240
p-value: 8.48E-16
t: -12.1230927
B: 25.690502
Gene: HIST1H2AG
Gene name: histone cluster 1, H2ag

Chart 1: HIST1H2AG is differentially expressed in triple negative breast cancer.

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.

Rank: 2331
Probe ID: 2899756
p-value: 1.98E-06
t: -5.37
B: 4.527366
Gene: HIST1H2AG
Gene name: histone cluster 1, H2ag

Chart 2: HIST1H2AG is differentially expressed in early-onset human breast cancer.

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: HIST1H2AG is expressed at significantly higher levels in primary TNBC tumor cells as compared to normal cells of the breast.

The mRNA expression level of HIST1H2AG in normal mammary ductal cells (left) and in the isolated primary tumor cells of patients with triple negative breast cancer (right) is graphically depicted with the result of a statistical test evaluating significance of difference in HIST1H2AG expression between normal cells of the breast and TNBC tumor cells, a *p*-value, listed above.

Figure 2: HIST1H2AG expression correlates with post-progression survival in patients with basal-like breast cancer, but not in patients with luminal A, luminal B, HER2+, or normal-like subtype cancer.

Depicted in this Kaplan-Meier plot is the probability of post-progression survival (PPS) for $n=77$ patients with basal subtype breast cancer (upper left), $n=92$ patients with HER2+ breast cancer (upper right), $n=128$ patients with luminal A breast cancer (below left), and $n=148$ patients with luminal B breast cancer (below right), stratified into two groups, based on low or high expression of HIST1H2AG in patient primary tumors. The log rank p -value denoting statistical significance of difference in post-progression 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 HIST1H2AG 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.

1 Low HIST1H2AG expression: 17.16 months

2 High HIST1H2AG expression: 33.7 months

3 **Chart 3: Median post-progression survival is superior in basal-like subtype breast cancer patients**
4 **with high primary tumor expression of HIST1H2AG.**

5 The post-progression survival of $n=77$ basal-like subtype breast cancer patients based on stratification into
6 low or high tumor expression of HIST1H2AG is listed in this chart.

Figure 3: HIST1H2AG expression correlates with recurrence-free survival in triple negative breast cancer patients whose tumors are of the basal-like 1 and luminal androgen receptor molecular subtype.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival (RFS) for $n=251$ patients with basal-like 1 subtype TNBC (upper left), $n=101$ patients with basal-like 2 subtype TNBC (upper middle), $n=300$ patients with immunomodulatory subtype TNBC (upper right), $n=253$ patients with luminal androgen receptor subtype TNBC (lower left), $n=211$ patients with mesenchymal subtype TNBC (lower middle), and $n=81$ patients with mesenchymal stem-like subtype TNBC (lower right), stratified into two groups, based on low or high expression of HIST1H2AG in the primary tumors of patients with TNBC. 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 HIST1H2AG 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.

Basal-like 1 subtype

Low HIST1H2AG expression: 25.2 months

High HIST1H2AG expression: 19 months

Luminal androgen receptor subtype

Low HIST1H2AG expression: 27 months

High HIST1H2AG expression: 15 months

Chart 4: In patients with basal-like 1 and luminal androgen receptor subtype disease, median recurrence-free survival is superior in triple negative breast cancer patients with low primary tumor expression of HIST1H2AG.

The recurrence-free survival of $n=251$ basal-like 1 subtype TNBC patients and $n=253$ luminal androgen receptor subtype TNBC patients based on stratification into low or high tumor expression of HIST1H2AG is listed in this chart.