Differential expression of histone cluster 1, H2bh 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, H2bh, HIST1H2BH, when comparing the tumor cells of patients with triple negative breast cancer to normal mammary ductal cells². HIST1H2BH was also differentially expressed in the brain metastases of patients with metastatic breast cancer³. HIST1H2BH 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 HIST1H2BH in primary tumors of the breast was correlated with overall survival in patients with luminal A type cancer, while within triple negative breast cancer, primary tumor expression of HIST1H2BH was correlated with distant metastasis-free survival in patients with immunomodulatory subtype disease. HIST1H2BH may be of relevance to initiation, maintenance or progression of triple negative breast cancers.

Keywords: breast cancer, HIST1H2BH, histone cluster 1, H2bh, 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 GSE52604³ 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. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer; analysis was performed using platform GPL6480. 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 HIST1H2BH 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 HIST1H2BH mRNA expression levels with overall survival (OS) in $n=431$ patients with basal subtype cancer, $n=596$ patients with luminal A subtype cancer, $n=439$ patients with luminal B subtype cancer, $n=362$ patients with HER2+ cancer, and $n=51$ patients with normal-like subtype cancer, in the lower quartile. In patients with triple negative breast cancer⁷, we analyzed distant metastasis-free survival (DMFS) for $n=171$ patients with basal-like 1 subtype TNBC, $n=71$ patients with basal-like 2 subtype TNBC, $n=219$ patients with immunomodulatory subtype TNBC, $n=138$ patients with luminal androgen receptor subtype TNBC, $n=130$ patients with mesenchymal subtype TNBC, and $n=39$ 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}.

HIST1H2BH 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, H2bh, encoded by HIST1H2BH, 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, HIST1H2BH ranked 85 out of 45015 total transcripts, equating to 99.8% differential expression (Chart 1). Differential expression

of HIST1H2BH in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 1; $p=4.97E-13$).

Analysis of a second microarray dataset³, here studying global gene expression patterns in the brain metastases of patients with metastatic breast cancer, again revealed significant differential expression of HIST1H2BH in tumor tissue of patients with human breast cancer (Chart 2). When sorting each of the genes expressed in the brain metastases of patients with breast cancer based on significance of difference as compared to normal breast tissue, HIST1H2BH ranked 6594 out of 41093 total transcripts, equating to 84.0% differential expression (Chart 2). Differential expression of HIST1H2BH in the brain metastases of patients with breast cancer was statistically significant (Chart 2; $p=7.58E-04$).

These data suggested that differential expression of HIST1H2BH was not an artifact of a single microarray dataset, nor was it strictly associated with primary tumors of the breast, rather a reflection of the spectrum of transformation in human cancer.

HIST1H2BH 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 HIST1H2BH - 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 HIST1H2BH expression change in TNBC. HIST1H2BH 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 HIST1H2BH in primary tumor-isolated triple negative breast cancer cells was statistically significant (Figure 1: $p<0.0001$). HIST1H2BH was expressed at 218.9 ± 184.6 arbitrary units (A.U.) in normal mammary ductal cells, while it was expressed at 1198.9 ± 645.5 A.U. in tumor cells of patients with triple negative breast cancer. We calculated a mean fold change of 5.48 when comparing triple negative breast cancer cells to normal mammary ductal cells.

HIST1H2BH expression correlates with survival outcomes in luminal A human breast cancer.

We performed Kaplan-Meier survival analysis to evaluate potential relationships between primary tumor HIST1H2BH 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+, normal-like, and basal-like. We observed a statistically significant correlation between HIST1H2BH expression and overall survival (OS) in patients with luminal A subtype breast cancer, in the lower quartile (Figure 2; log rank p -value: 0.034 for overall survival, hazard ratio: 1.81 (1.04-3.16) (Fig. 2)).

HIST1H2BH mRNA levels were a negative prognostic indicator in luminal A breast cancer patients. Median OS was 191.21 months for luminal A patients with low tumor expression of HIST1H2BH while median OS was 173.72 months for luminal A patients with high tumor expression of HIST1H2BH (Chart 3).

HIST1H2BH primary tumor expression was not correlated with overall survival in basal subtype (Figure 2; log rank p -value: 0.71 for OS, hazard ratio: 0.92 (0.6-1.42) (Fig. 2), luminal B subtype (Figure 2; log rank p -value: 0.97 for OS, hazard ratio: 0.99 (0.66-1.49) (Fig. 2)), in HER2+ breast cancer (Figure 2; log rank p -value: 0.1 for OS, hazard ratio: 1.49 (0.92-2.43) (Fig. 2)), or in the normal-like subtype (Figure 2; log rank p -value: 0.64 for OS, hazard ratio: 0.78 (0.27-2.25) (Fig. 2)).

Influence of primary tumor HIST1H2BH expression on survival outcomes based on TNBC molecular subtype.

We also performed Kaplan-Meier survival analysis to examine relationships between HIST1H2BH mRNA expression levels with survival strictly in patients with triple negative breast cancer, analyzing outcomes based on the TNBC molecular subtype⁸ of the patient: basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor. We observed a statistically significant correlation between HIST1H2BH expression and distant metastasis-free survival (DMFS) in TNBC patients with immunomodulatory subtype disease, in the upper tertile (Figure 3; log rank *p*-value: 0.077 for distant metastasis-free survival, hazard ratio: 0.56 (0.3-1.07) (Fig. 3)). HIST1H2BH mRNA levels were a positive prognostic indicator in patients with immunomodulatory TNBC. DMFS was, on average, markedly shorter for immunomodulatory TNBC patients with low tumor expression of HIST1H2BH as compared to immunomodulatory TNBC patients with high tumor expression of HIST1H2BH (Chart 4).

HIST1H2BH primary tumor expression was not correlated with distant metastasis-free survival in the basal-like 1 subtype (Figure 3; log rank *p*-value: 0.6 for DMFS, hazard ratio: 0.85 (0.46-1.57) (Fig. 3)), in basal-like 2 subtype (Figure 3; log rank *p*-value: 0.6 for DMFS, hazard ratio: 1.24 (0.55-2.75) (Fig. 3)), in the mesenchymal subtype (Figure 3; log rank *p*-value: 0.51 for DMFS, hazard ratio: 1.23 (0.67-2.25) (Fig. 3)), in the mesenchymal stem-like subtype (Figure 3; log rank *p*-value: 0.96 for DMFS, hazard ratio: 0.97 (0.33-2.86) (Fig. 3)), or in the luminal androgen receptor subtype (Figure 3; log rank *p*-value: 0.11 for DMFS, hazard ratio: 0.58 (0.29-1.14) (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 differential expression of histone cluster 1, H2bh, encoded by the HIST1H2BH gene, is a defining transcriptional feature of triple negative breast cancer. HIST1H2BH was also differentially expressed in the brain metastases of patients with metastatic breast cancer. Expression of HIST1H2BH was correlated with overall survival in patients with luminal A subtype breast cancer, and within triple negative breast cancer patients, primary tumor expression of HIST1H2BH was correlated with distant metastasis-free survival in patients with immunomodulatory subtype disease. Histone cluster 1, H2bh 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. Salhia, B., Kiefer, J., Ross, J.T., Metapally, R., Martinez, R.A., Johnson, K.N., DiPerna, D.M., Paquette, K.M., Jung, S., Nasser, S. and Wallstrom, G., 2014. Integrated genomic and epigenomic analysis of breast cancer brain metastasis. *Plos one*, 9(1), p.e85448.
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.
8. Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y. and Pietenpol, J.A., 2011. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *The Journal of clinical investigation*, 121(7), pp.2750-2767.

Rank: 85
Probe ID: 22940
p-value: 4.97E-13
t: -10.0019047
B: 19.53118
Gene: HIST1H2BH
Gene name: histone cluster 1, H2bh

Chart 1: HIST1H2BH 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: 6594
Probe ID: A_23_P59069
p-value: 7.58E-04
t: -3.6027899
B: -0.966932
Gene: HIST1H2BH
Gene name: histone cluster 1, H2bh

Chart 2: HIST1H2BH is differentially expressed in brain metastasis in human metastatic 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: HIST1H2BH is expressed at significantly higher levels in primary TNBC tumor cells as compared to normal cells of the breast.

The mRNA expression level of HIST1H2BH 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 HIST1H2BH expression between normal cells of the breast and TNBC tumor cells, a *p*-value, listed above.

Figure 2: HIST1H2BH expression correlates with overall survival in patients with luminal A breast cancer, but not in patients with basal, luminal B, HER2+, or normal-like subtype cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=431$ patients with basal subtype breast cancer (upper left), $n=362$ patients with HER2+ breast cancer (upper right), $n=596$ patients with luminal A breast cancer (middle left), $n=439$ patients with luminal B breast cancer (middle right), and $n=51$ patients with normal-like breast cancer (below) stratified into two groups, based on low or high expression of HIST1H2BH in patient primary tumors, in the lower quartile. The log rank p -value denoting statistical significance of difference in overall 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 HIST1H2BH 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 HIST1H2BH expression: 191.21 months

2 High HIST1H2BH expression: 173.72 months

3 **Chart 3: Median overall survival is superior in luminal A subtype breast cancer patients with low**
4 **primary tumor expression of HIST1H2BH.**

5 The overall survival of $n=596$ luminal A subtype breast cancer patients based on stratification into low or
6 high tumor expression of HIST1H2BH is listed in this chart, in the lower quartile.

Figure 3: HIST1H2BH expression correlates with distant metastasis-free survival in triple negative breast cancer patients whose tumors are of the immunomodulatory molecular subtype.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival (DMFS) for $n=171$ patients with basal-like 1 subtype TNBC (upper left), $n=71$ patients with basal-like 2 subtype TNBC (upper right), $n=219$ patients with immunomodulatory subtype TNBC (middle left), $n=138$ patients with luminal androgen receptor subtype TNBC (middle right), $n=130$ patients with mesenchymal subtype TNBC (lower left), and $n=39$ patients with mesenchymal stem-like subtype TNBC (lower right), stratified into two groups, based on low or high expression of HIST1H2BH in the primary tumors of patients with TNBC, in the upper tertile. The log rank p-value denoting statistical significance of difference in distant metastasis-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 HIST1H2BH 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.