A single nucleotide variant on chromosome 10 residing within ITIH2 distinguishes patients with basal-like human breast cancer.

Shahan Mamoor, MS
shahanmamoor@gmail.com
East Islip, NY 11730

Patients diagnosed with basal-like breast cancer face a more aggressive disease course and more dismal prognosis than patients diagnosed with luminal A and luminal B breast cancer molecular subtypes (1-4). We mined published microarray data (5, 6) to understand in an unbiased fashion the most distinguishing genetic and transcriptional features of tumors from patients with basal or basal-like subtype breast cancer. We identified a single nucleotide polymorphism, rs2298319, residing within ITIH2 as among the most significant genetic differences in the tumors of patients with basal-like breast cancer. In a separate cohort of patients with basal subtype breast cancer, we observed transcriptome-wide differential tumor expression of a ITIH2 transcript. Analysis of patient survival data revealed that ITIH2 primary tumor expression was correlated with distant metastasis-free survival in patients with basal-like breast cancer. Sequence variation in the ITIH2 gene may be important in understanding differences in genetic background that favor development of basal subtype human breast cancer.

Keywords: basal subtype, basal-like breast cancer, BLBC, ITIH2, systems biology of breast cancer, targeted therapeutics in breast cancer, breast cancer, triple negative breast cancer.

Classification of disease types and disease subtypes enables development of enhanced diagnostic capabilities, prognostic information, as well as a nuanced understanding of human disease that can support superior medical, surgical and radiological treatment of complex diseases such as cancer. Molecular subtypes in human breast cancer include luminal A, luminal B, basal or basal-like, and HER2+. Systems-level analyses, such as comparative tumor transcriptome analyses, can inform such approaches. Importantly, differences in genetic background that can influence disease frequency or progression in basal subtype (1, 2) or triple negative breast cancer (which shares significant overlap with basal subtype breast cancer (3, 4)) are difficult to discern without whole-genome sequencing data. However, in the case of whole genome sequencing, data indicating that changes at the level of the genome at single nucleotide resolution are accompanied by differences in gene expression, between molecular subtypes and at the level of the transcriptome, are still lacking, but could be ascertained based on combined analysis of microarray data that encompasses both transcript and SNP-level data. We mined published microarray data (5, 6) to identify the most significant genetic and transcriptional differences when comparing human breast cancer molecular subtypes. Integrative microarray dataset analysis has provided data illuminating genes whose sequence and expression defines basal-like subtype breast cancers, and we present evidence here.

Methods

We utilized datasets GSE10893 (5) and GSE87049 (6) for this global differential gene expression analysis of basal subtype human breast cancer in conjunction with GEO2R. GSE10893 was generated using Illumina Human-1 Genotyping BeadChip technology with $n=37$ basal-like breast tumors and $n=132$ primary tumors of the breast from patients diagnosed with breast cancer of different, non-basal molecular subtypes (luminal A, luminal B, HER2+, claudin, claudin-low, normal-like); analysis was performed using platform GPL8864. GSE87049 was generated using Affymetrix Human Gene 1.0 ST Array [transcript (gene) version] technology with $n=14$ basal-like breast tumors and $n=109$ breast tumors of other molecular subtypes (luminal A, luminal B, HER2-enriched, and normal-like); analysis was performed using platform GPL6244. 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 ITIH2 gene expression was significantly different between the tumors of patients with basal and non-basal molecular subtype breast cancer using a two-tailed t-test with Welch's correction. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool (8) for correlation of ITIH2 mRNA expression levels with distant metastasis-free survival in $n=2765$ breast cancer patients, $n=630$ basal subtype breast cancer patients, $n=998$ luminal A subtype breast cancer patients, $n=673$ luminal B subtype breast cancer patients, $n=401$ HER2+ subtype breast cancer patients, and $n=63$ normal subtype breast cancer patients.

Results

SNP array genotyping identifies rs2298319 as a defining sequence variation in human basal breast cancer.

We used published microarray data (5) to compare the whole tumor genomes of 37 patients with basal or basal-like subtype breast cancer, with that of 132 patients with disease of other molecular subtype, including luminal A, luminal B, HER2+, claudin, claudin-low and normal-like, to identify single nucleotide polymorphisms that describe the most significant variations in the tumor genomes of patients with basal-like subtype breast cancer (BLBC).

SNP genotyping arrays utilize large differences in signal, after probe hybridization to DNA rather than RNA, to identify polymorphic sites in the genome at single nucleotide resolution. We identified a single nucleotide polymorphism, rs2298319, residing within the ITIH2 gene on chromosome 10 (10p14)

as among the most significant genetic differences in the tumors of patients with basal subtype breast cancer as compared to that of other molecular subtypes. Polymorphism at rs2298319 was the 75th most significant difference, genome-wide, when comparing the tumor genomes of basal subtype patients with that of tumors from patients with other molecular subtypes (Rank: 75 out of 108250 total SNPs, equating to 99.9% (p -value: $1.09\text{E-}06$)).

ITIH2 is differentially expressed in basal-like subtype human breast cancer.

Analysis of a second published microarray dataset, comparing tumor transcriptome data from a separate cohort of Mexican patients with basal subtype breast cancer (14 tumors from patients with basal-like breast cancer and 109 tumors from patients with breast cancer of other molecular subtype [luminal A, luminal B, HER2-enriched, and normal-like]) (6) revealed transcriptome-wide differential expression of a ITIH2 transcript in patients with basal subtype human breast cancer (Table 2). When sorting each of the transcripts whose expression was measured globally, ITIH2 expression ranked 15043 out of 33297 total transcripts, equating to 54.8% differential expression; this was not statistically significant (p -value: $1.80\text{E-}01$).

ITIH2 expression correlates with distant metastasis-free survival in basal subtype breast cancer patients.

We asked whether primary tumor expression of ITIH2 was correlated with distant metastasis-free survival outcomes in patients with breast cancer when analyzing based on PAM50 molecular subtype. We observed a statistically significant correlation between primary tumor expression of ITIH2 and distant metastasis-free survival (DMFS) in patients with basal subtype human breast cancer (Figure 2; p -value: 0.02; hazard ratio: 1.42 (1.05-1.91)). ITIH2 primary tumor expression was a negative prognostic indicator with respect to distant metastasis-free survival in patients with basal subtype cancer. Patients with low tumor expression of ITIH2 possessed median DMFS of 53.45 months, while patients with high tumor expression of ITIH2 possessed median DMFS of 27.04 months.

ITIH2 primary tumor expression was not correlated with distant metastasis-free survival in all patients (Figure 2; p -value: 0.77; hazard ratio: 1.02 (0.88-1.19)), in patients with luminal A subtype breast cancer (Figure 2; p -value: 0.79; hazard ratio: 1.04 (0.76-1.45)), in patients with luminal B subtype breast cancer (Figure 2; p -value: 0.14; hazard ratio: 1.25 (0.93-1.68)), in patients with HER2+ subtype breast cancer (Figure 2; p -value: 0.7; hazard ratio: 0.93 (0.66-1.33)), and in patients with normal subtype breast cancer (Figure 2; p -value: 0.95; hazard ratio: 0.97 (0.36-2.59)).

Discussion

We found through analysis of published microarray datasets (5, 6) that a genomic position marked by SNP rs2298319 was among the most significant sequence variations in the tumor genomes of patients with basal subtype human breast cancer when compared with that of patients with breast cancer of other molecular subtypes. rs2298319 resides within the ITIH2 gene. Similar comparative tumor transcriptome study based on molecular subtype in a separate patient dataset revealed differential expression of a ITIH2 transcript when comparing tumors of other molecular subtypes against that of tumors from patients of the basal subtype. Kaplan-Meier analysis of patient survival data revealed that ITIH2 expression was negatively correlated with distant metastasis-free survival in patients with basal-like breast cancer. Changes in ITIH2 expression may be important events to understand as part of the myriad processes that likely govern transformation and tumor maintenance in basal or basal-like human breast cancer.

References

1. Rakha, E.A., Reis-Filho, J.S. and Ellis, I.O., 2008. Basal-like breast cancer: a critical review. *Journal of clinical oncology*, 26(15), pp.2568-2581.
2. Milioli, H.H., Tishchenko, I., Riveros, C., Berretta, R. and Moscato, P., 2017. Basal-like breast cancer: molecular profiles, clinical features and survival outcomes. *BMC medical genomics*, 10(1), pp.1-17.
3. 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.
4. 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.
5. Weigman, V.J., Chao, H.H., Shabalin, A.A., He, X., Parker, J.S., Nordgard, S.H., Grushko, T., Huo, D., Nwachukwu, C., Nobel, A. and Kristensen, V.N., 2012. Basal-like Breast cancer DNA copy number losses identify genes involved in genomic instability, response to therapy, and patient survival. *Breast cancer research and treatment*, 133(3), pp.865-880.
6. Romero-Cordoba, S.L., Salido-Guadarrama, I., Rebollar-Vega, R., Bautista-Piña, V., Dominguez-Reyes, C., Tenorio-Torres, A., Villegas-Carlos, F., Fernández López, J.C., Uribe-Figueroa, L., Alfaro-Ruiz, L. and Hidalgo-Miranda, A., 2021. Comprehensive omic characterization of breast cancer in Mexican-Hispanic women. *Nature communications*, 12(1), pp.1-19.
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: 75
SNP: rs2298319
p-value: 1.09E-06
t: 5.05170221
B: 5.219491
Chromosome: 10

Table 1: A genomic position marked by rs2298319 (residing within the ITIH2 gene) on chromosome 10 is among the most significant genetic differences in the tumor genomes of humans with basal-like breast cancer when compared with that of other molecular subtypes.

The rank of global differential expression, SNP ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, and *B*, the log-odds of differential expression between the groups compared are listed in this chart.

Figure 1: rs2298319 is significantly enriched in the primary tumors of patients with basal subtype human breast cancer.

A quantitative measure of ITIH2 polymorphism at rs2298319 in the tumors of patients with basal subtype breast cancer (left) and in the tumors of patients with breast cancer of non-basal molecular subtype (right) is graphically depicted; the result of a statistical test evaluating significance of difference in this signal between the tumors of basal subtype patients and the tumors of patients with cancer of other molecular subtype is $p < 0.0001$. Enrichment here indicates that in the basal subtype, probe signal was significantly increased as compared to other molecular subtypes considered together, demonstrating a difference in allelic frequency in basal subtype patients at this genomic site.

Rank: 15043
probe: 7926061
p-value: 1.80E-01
t: -1.3471285
B: -5.82414
Gene: ITIH2
Gene name: inter-alpha-trypsin inhibitor heavy chain 2

Table 2: ITIH2 is modestly differentially expressed in a separate cohort of patients with basal subtype breast cancer, though not statistically significantly so.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 2: ITIH2 primary tumor expression correlates with distant metastasis-free survival in basal-like subtype breast cancer patients.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival for $n=2765$ breast cancer patients (above, left), $n=630$ basal subtype breast cancer patients (above, middle), $n=401$ HER2+ subtype breast cancer patients (above, right), $n=998$ luminal A subtype breast cancer patients (below, left), $n=673$ luminal B subtype breast cancer patients (below, middle), and $n=63$ normal subtype breast cancer patients (below, right) stratified into two groups, based on low or high expression of ITIH2 in patient primary tumors. 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 ITIH2 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 subtype

Low ITIH2 expression: 53.45 months

High ITIH2 expression: 27.04 months

Table 3: Median DMFS is superior in basal subtype breast cancer patients with low primary tumor expression of ITIH2.

The median DMFS (distant metastasis-free survival) of $n=630$ basal subtype breast cancer patients with low primary tumor expression of ITIH2 and high primary tumor expression of ITIH2 is listed in this chart.