A single nucleotide variant on chromosome 5 residing within NLN distinguishes patients with basal-like human breast cancer.

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Patients diagnosed with basal-like (also known as basal) subtype 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, rs3733657, residing within NLN, 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 expression of a NLN transcript. Analysis of patient survival data revealed that NLN primary tumor expression was correlated with recurrence-free survival in patients of every molecular subtype. Sequence variation in the NLN 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, 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 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 NLN 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 NLN mRNA expression levels with recurrence-free survival in $n=2032$ breast cancer patients, $n=442$ basal subtype breast cancer patients, $n=631$ luminal A subtype breast cancer patients, $n=566$ luminal B subtype breast cancer patients, $n=358$ HER2+ subtype breast cancer patients, and $n=35$ normal subtype breast cancer patients.

Results

SNP array genotyping identifies rs3733657 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 hybridization to DNA rather than RNA, to identify polymorphic sites in the genome at single nucleotide resolution. We identified a single nucleotide polymorphism (rs3733657) residing within the NLN gene, on chromosome 5 (5q12.3), 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 rs3733657 was the 83rd 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: 83 out of 108250 total SNPs, equating to 99.9% (p -value: $1.23\text{E-}06$)).

NLN 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 NLN transcript in patients with basal subtype human breast cancer (Table 2). When sorting each of the transcripts whose expression was measured globally, NLN expression ranked 3134 out of 33297 total transcripts, equating to 90.6% differential expression; this was statistically significant (p -value: $2.12\text{E-}04$).

NLN expression correlates with patient survival in human breast cancer.

We asked whether primary tumor expression of NLN was correlated with patient survival outcomes in patients with breast cancer when analyzing outcomes based on PAM50 molecular subtype. We observed a correlation between primary tumor expression of NLN and recurrence-free survival (RFS) in patients with human breast cancer (all molecular subtypes considered together) (Figure 2; p -value: 0.0074; hazard ratio: 0.81 (0.71-0.95)). NLN primary tumor expression was a positive prognostic indicator in patients with breast cancer. Patients with low tumor expression of NLN possessed median RFS of 34.8 months, while patients with high tumor expression of NLN possessed median RFS of 43 months.

We also observed a correlation between primary tumor expression of NLN and recurrence-free survival (RFS) in patients with basal subtype human breast cancer (Figure 2; p -value: 0.05; hazard ratio: 0.74 (0.54-1)). NLN primary tumor expression was a positive prognostic indicator in patients with basal subtype cancer. Patients with low tumor expression of NLN possessed median RFS of 18.35 months, while patients with high tumor expression of NLN possessed median RFS of 21.82 months.

We also observed a correlation between primary tumor expression of NLN and recurrence-free survival (RFS) in patients with luminal B subtype human breast cancer (Figure 2; p -value: 0.082; hazard ratio: 0.79 (0.61-1.03)). NLN primary tumor expression was a positive prognostic indicator in patients with luminal B subtype cancer. Luminal B patients with low tumor expression of NLN possessed median RFS of 30.72 months, while luminal B patients with high tumor expression of NLN possessed median RFS of 39 months.

We also observed a correlation between primary tumor expression of NLN and recurrence-free survival (RFS) in patients with HER2+ subtype human breast cancer (Figure 2; p -value: 0.08; hazard ratio: 0.75 (0.54-1.04)). NLN primary tumor expression was a positive prognostic indicator in patients with HER2+ subtype cancer. HER2+ patients with low tumor expression of NLN possessed median RFS of 105.6 months, while HER2+ patients with high tumor expression of NLN possessed median RFS of 171.43 months.

In summary, NLN was correlated with recurrence-free survival in patients with basal-like breast cancer, and in patients with luminal B breast cancer and HER2+ breast cancer.

Discussion

We found through analysis of published microarray datasets (5, 6) that a genomic position marked by SNP rs3733657 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. rs3733657 resides within the NLN gene. Similar comparative tumor transcriptome study based on molecular subtype in a separate patient dataset revealed differential expression of a NLN 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 NLN expression was negatively correlated with recurrence-free survival in basal-like breast cancer, as well as in patients with luminal B and HER2+ breast cancer. Changes in NLN 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

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Rank: 83
SNP: rs3733657
p-value: 1.23E-06
t: -5.02629873
B: 5.114475

Table 1: A genomic position marked by rs3733657 (residing within the NLN gene) is among the most significant genetic differences in the tumor genomes of basal subtype human 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: rs3733657 is significantly depleted in the primary tumors of patients with basal subtype human breast cancer.

A quantitative measure of NLN polymorphism at rs3733657 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 $p<0.0001$. Depletion here indicates that in the basal subtype, probe signal was significantly decreased as compared to other molecular subtypes considered together, demonstrating a difference in allelic frequency in basal subtype patients at this genomic site.

Rank: 3134
probe: 8105663
p-value: 2.12E-04
t: 3.816844
B: 0.11981
Gene: NLN
Gene name: neurolysin

Table 2: NLN is among the genes most differentially expressed, transcriptome-wide, in a separate cohort of patients with basal subtype breast cancer.

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: NLN primary tumor expression correlates with recurrence-free survival in each molecular subtype in human breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=2032$ breast cancer patients (above, left), $n=442$ basal subtype breast cancer patients (above, middle), $n=358$ HER2+ subtype breast cancer patients (above, right), $n=631$ luminal A subtype breast cancer patients (below, left), $n=566$ luminal B subtype breast cancer patients (below, middle), and $n=83$ normal subtype breast cancer patients (below, right) stratified into two groups, based on low or high expression of NLN 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 NLN 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 NLN expression: 18.35 months

High NLN expression: 21.82 months

Luminal B subtype

Low NLN expression: 30.72 months

High NLN expression: 39 months

HER2+ subtype

Low NLN expression: 105.6 months

High NLN expression: 171.43 months

Median RFS is superior in basal, luminal B, and HER2+ subtype breast cancer patients with high primary tumor expression of NLN.

The median RFS (recurrence-free survival) of $n=442$ basal subtype, $n=566$ luminal B subtype and $n=358$ HER2+ subtype breast cancer patients with low primary tumor expression of NLN and high primary tumor expression of NLN is listed in this chart.