Keratin 19 expression correlates with poor prognosis in breast cancer

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Keratin 19 expression correlates with poor prognosis in breast cancer

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Abstract:

Breast cancer expression profiling has been used for determining biomarkers. Using gene expression profiles of 2400 patients we identified keratin 19 (KRT19) as a highly deregulated gene in breast cancer. KRT19 expression is independent of patient race but correlates with disease grade, and ER, PR or HER2 expression. Expression of TPD52, GATA3 and KRT18 was increased in KRT19 expressing patients. Furthermore, KRT19 expression was associated with ER up-regulation and Luminal B gene signatures, as well as a constitutive RAF1 signaling pathway. Finally, KRT19 expression correlated with poor overall survival. Taken together, our results suggest that KRT19 expression can be used as a prognostic marker.

Keywords: Cytokeratin 19, CK19, KRT19, Microarray.

1. Introduction:

Despite considerable improvements in the overall survival over the past decades, breast cancer is classified as one of the leading causes of cancer death in women [1]. Breast cancer is a complex and heterogeneous diseases displaying considerable variation in its clinical and molecular characteristics, and thus this cancer remains a challenge of management. Understanding of the detailed molecular profiles of individual patients will provide an opportunity for a personalized treatment approach. Recent development of high-throughput technologies to study genetic changes brought us close to this goal.

For a long time many investigators have used high-throughput analysis of patient materials to understand the genetic bias in breast cancer [2-5]. Microarray analysis of RNA expression allows us to understand the complete gene expression pattern in individual tumors. Numerous microarray studies have focused on selected patients or disease groups and identified different biomarkers [1,4,3]. In this report we aimed to analyze the mRNA expression profiles of a large set of breast cancer patients. We provide evidence that expression of KRT19 has significant influence on disease progression and thus can be used as a molecular tool to classify breast cancer patients.

2. Materials and methods:

2.1. Expression data from patient samples:

As a source of primary patient material, we collected mRNA expression data from breast cancer patient samples. The breast cancer data set GSE9574, GSE15852 and GSE16873 were downloaded from NCBI Gene Expression Omnibus (GEO). These data sets include mRNA expression data from 69 cancer patients and corresponding tissue samples from 70 healthy donors. All three data sets are from the same platform, Affymetrix Human Genome U133A Array (GPL96). We used raw CEL files and processed by RMA normalization. Normalized data were then analyzed for mRNA expression differences [6-8].

2.2. Correlation analysis:

We then used the raw CEL files from 2331 breast cancer patient samples. Raw data were normalized using RMA normalization and then used for correlation analysis. Linear regression analysis incorporated in GraphPad Prism five was used to check correlation.

2.3. Gene sets enrichment analysis:

Normalized data from 2331 patient samples were used. Expression data were sorted for highest and lowest KRT19 expression. We used data from100 patients with higher KRT19 expression and data from 100 patients with lower KRT19 expression for gene set enrichment analysis (GESA).

2.4. Prognosis and survival analysis:

Two data sets with higher or lower KRT19 expression from 2331 patients were analyzed to check the importance of KRT19 for overall survival of the patients.

3. Results:

3.1. KRT19 is upregulated in breast cancer:

We first set out to identify deregulated genes from heterogeneous breast cancer patient samples. As a source of primary patient material three data sets GSE9574 [9], GSE15852 [10] and GSE16873 [11] were analyzed. These datasets include mRNA expression data from 70 normal breast tissue samples and 69 cancer tissue samples. After processing raw data with RMA normalization, data were subjected to ANOVA test. Using a twofold change in expression as a cut off value we identified 12 upregulated and 17 downregulated genes (Fig. 1). We then clustered these genes using Functional Annotation Clustering function incorporated in DAVID. We observed that upregulated genes showed enrichment (score 2.01) in "response to estrogen stimulus" (p= 0.0021, Benjamini=0.56) and "response to steroid hormone stimulus"

(p= 0.0068, Benjamini=0.74) biological functions. Since KRT19 displayed the highest fold-change (Fold-change=8.38, p=0.003224) as well as is involved in estrogen and hormone responses we intended to identify the importance of this protein in breast cancer.

3.2. KRT19 expression correlates with tumor grade, ER, PR and HER2 expression:

The risk and prognosis of breast cancer are classically estimated by several factors including tumor grade, estrogen receptor (ER) and progesterone receptor (PR) status, HER2 (ERBB2) overexpression , etc. [12]. We used 2331 breast cancer patient samples to define these factors. To assess whether KRT19 expression has a role in breast cancer tumor grade, we compared KRT19 expression in three different patient groups. We observed that, while grade 1 and grade 2 patient groups express equal level of KTR19, the grade 3 patient group expresses significantly less KRT19 (Fig. 1B, first panel). Heterogeneity of different patient groups is a common phenomenon in cancer. Although KRT19 expression is altered in tumors of different grades, the difference in KTR19 was negligible between patient races (Fig. 1B, second panel) and between different ages (data not shown). A comparison within the ER negative and ER positive patient groups suggests that KRT19 expression represents a group of patients that are ER positive (Fig. 1B, third panel). Similar expression patterns were observed in the PR negative and PR positive patient groups (Fig. 1B fourth panel) as well as in the HER2 negative and HER2 positive patient groups ((Fig. 1B, fifth panel).

3.3. KRT19 expression correlates with up-regulated genes

Since we observed that 12 genes are up-regulated and 17 genes are downregulated in breast cancer, we checked whether expression of the up-regulated genes correlate with that of KRT19. We plotted deregulated gene expression against KRT19 expression from 2331 patient samples data. We observed that the expression of up-regulated genes is mainly correlated with KRT19 expression (Fig. 2). TPD52, GATA3 and KRT18 displayed considerable correlation with KRT19. TPD52 is a tumor protein D52 family member that plays important roles in tumor progression [13]. GATA3 is a well-studied

transcription factor in breast cancer. Higher GATA3 expression has been reported to be associated with more aggressive breast cancer [14].

3.4. Higher KRT19 expression contributes to enrichment in ER positive signaling pathway:

The observation that KRT19 expression correlates with multiple genes functioning as markers of aggressive cancer, we hypothesized that KRT19 expression might correlate with certain breast cancer pathways. To check our hypothesis we ranked patients according to KRT19 higher and lower expression (Fig. 3A). Then we analyzed these two patient groups using GESA. Gene sets database c2.all.v.4.0 was used to check enrichment. We observed a significant enrichment in ER positive breast cancer (Fig. 3B) and Luminal B up-regulated (Fig. 3C) pathways of higher KRT19 expression.

3.5. Enrichments in Gene Ontology (GO) terms and in oncogenic signature

Then enrichment in GO terms was analyzed. Leading enrichments in GO biological processes were observed in mitochondrion organization and biogenesis (Fig. 4A), and in RNA processing (Fig. 4B). While enrichments in GO molecular function were evident in hormone receptor binding (Fig. 4C) and in translation initiation factor activity (Fig. 4D), enrichments in GO cellular compartments were mainly in the mitochondrion (Fig. 4E) and in mitochondrial membrane part (Fig. 4F). Furthermore, analysis in oncogenic signatures enrichments provided evidence of up-regulation of constitutive RAF1 signaling (Fig. 5A) as well as signaling regulated by EGFR activation (Fig. 5B).

3.6. Higher KRT19 expression correlates with poor prognosis

Since we observed that KRT19 expression is higher in ER, PR or HER2 positive breast cancer patients and correlates with ER positive signaling pathway, we hypothesized that KRT19 expression might correlate patient overall survival. We divided patients in two groups. One group patients carried lower level of KRT19, while other groups carried a higher level of KRT19. We observed that patients that

had higher KRT19 expression displayed poor relapse-free survival (Fig. 6A) as well as poor overall survival (Fig. 6B).

4. Discussion

The Keratin 19 (KRT19) (also known as K19, CK19, K1CS or Cytokeratin 19) is a member of the keratin family of proteins. Members of the keratin family of proteins are involved in regulating the structural integrity of epithelial cells. This family consists of more than 20 different proteins which are subdivided into two subfamilies, basic type II and acidic type I. Basic type keratins include KRT1 to KRT8, while acidic type keratins include KRT9 to KRT20. Besides maintaining structural integrity of epithelial cells, keratins play significant roles in cell signaling, stress response and apoptosis [15]. Moreover, the expression of keratins depends on the epithelial cell type and also correlates with a degree of epithelial cell differentiation [16]. Keratins undergo multiple post-translation modifications. During apoptosis KTR19 is cleaved by caspase 3, and soluble fractions are detectable in cancer patients [17]. By comparing mRNA expression data from breast cancer patients with that of normal tissues from healthy donors, we identified KRT19 as a highly upregulated gene.

KRT19 has been used to distinguish malignant tumors from benign tumors. KRT19 expression was reported to be upregulated in HER2 positive breast cancer [18]. HER2 is a receptor tyrosine kinase [19]. Our results also support the previous finding that KRT19 expression is enhanced in HER2 positive breast cancer patients. In addition, we report that KRT19 expression is also upregulated in tumors that express ER and PR. These findings suggest that elevated KRT19 expression is associated with a more aggressive breast cancer. Upregulation of the tumor associated proteins TPD52 and GATA3 in highly KRT19 expressing patients further supports this idea. Gene enrichment in ER positive and luminal B upregulated breast cancer pathways in higher KRT19 expressing patients suggests that KRT19 expression correlates with mainly ER positive breast cancers. The finding that higher KRT19 expression related to decreased relapse free survival as well as overall survival indicates that KRT19 expression has prognostic

value in breast cancer. In conclusion, data presented here suggest a potential role of KRT19 that can be used as an indicator of breast cancer prognosis.

Conflict of Interest Statement: The authors declare no conflict of interest.

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Figure legends:

Figure 1:

(A) Upregulated and downregulated genes in breast cancer compared to corresponding tissues. (B) KRT19 expression in different breast cancer tumor grades, races and ER, PR or HER2 positive or negative breast cancers.

Figure 2:

Correlation in between KRT19 expression and up-regulated or downregulated gene expression in breast cancer.

Figure 3:

(A) KRT19 expression in two different breast cancer patient groups. (B) Gene enrichment in breast cancer ER positive signaling pathway. (C) Gene enrichment in breast cancer luminal B signaling pathway.

Figure 4:

Gene enrichment in different GO biological processes (A and B), in GO molecular functions (C and D) and in GO cellular compartments (E and F).

Figure 5:

Gene enrichment in oncogenic signature. Higher KRT19 expression correlated with RAF upregulation (A) and RNA processing (B) signatures.

Figure 6:

(A) Relapse free survival and (B) overall survival in patients expressing higher and lower KRT19.

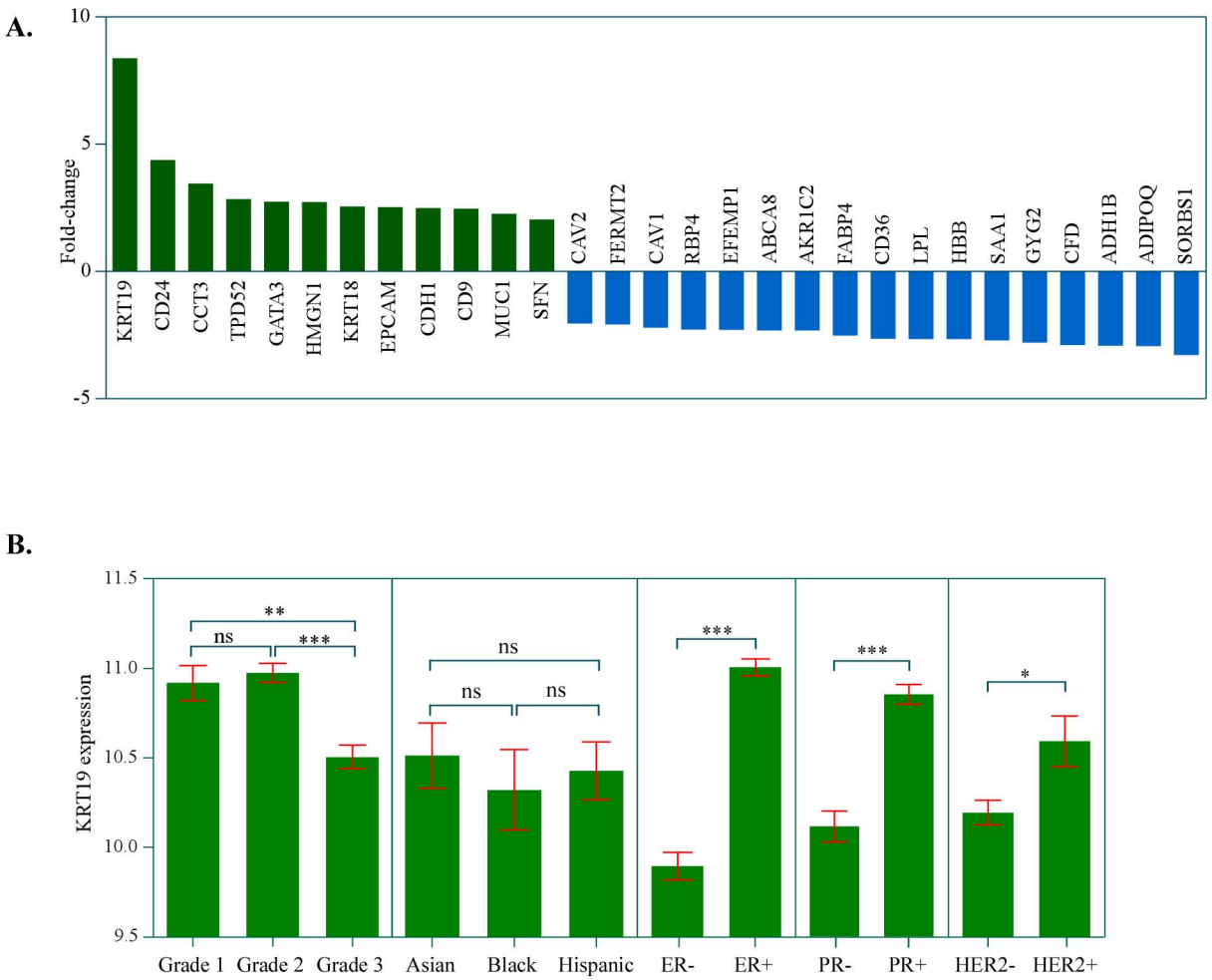
A.
Fold-change
10
5
0
-5
KRT19
CD24
CCT3
TPD52
GATA3
HMGN1
KRT18
EPCAM
CDH1
CD9
MUC1
SFN
CAV2
FERMT2
CAV1
RBP4
EFEMP1
ABCA8
AKR1C2
FABP4
CD36
LPL
HBB
SAA1
GYG2
CFD
ADH1B
ADIPOQ
SORBS1
B.
KRT19 expression
11.5
11.0
10.5
10.0
9.5
**
ns
***
ns
ns
ns
***
***
*
Grade 1
Grade 2
Grade 3
Asian
Black
Hispanic
ER-
ER+
PR-
PR+
HER2-
HER2+

Figure 1

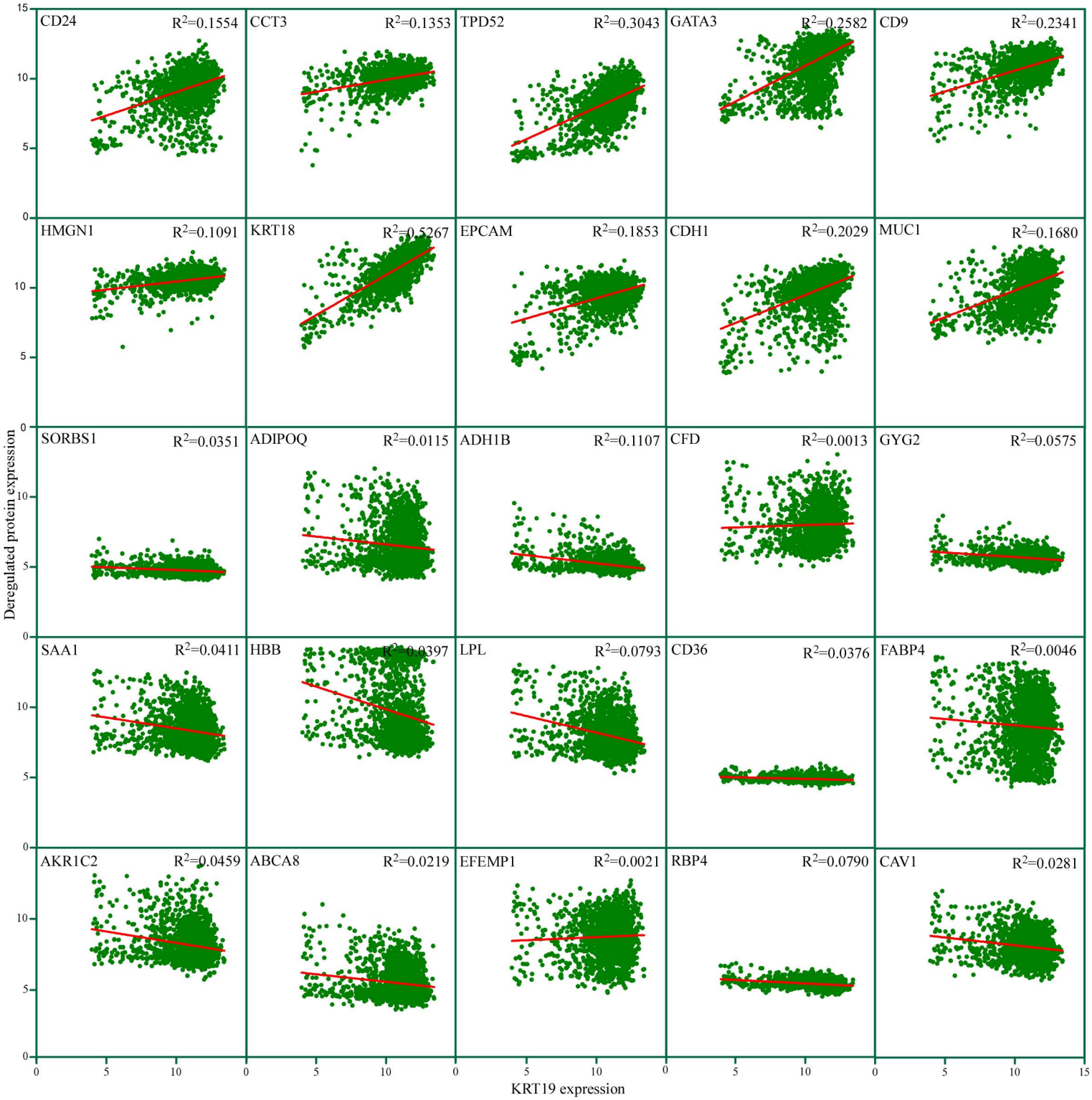
CD24 R2=0.1554
CCT3 R2=0.1353
TPD52 R2=0.3043
GATA3 R2=0.2582
CD9 R2=0.2341
HMGN1 R2=0.1091
KRT18 R2=0.5267
EPCAM R2=0.1853
CDH1 R2=0.2029
MUC1 R2=0.1680
SORBS1 R2=0.0351
ADIPOQ R2=0.0115
ADH1B R2=0.1107
CFD R2=0.0013
GYG2 R2=0.0575
SAA1 R2=0.0411
HBB R2=0.0397
LPL R2=0.0793
CD36 R2=0.0376
FABP4 R2=0.0046
AKR1C2 R2=0.0459
ABCA8 R2=0.0219
EFEMP1 R2=0.0021
RBP4 R2=0.0790
CAV1 R2=0.0281
Deregulated protein expression
KRT19 expression

Figure 2

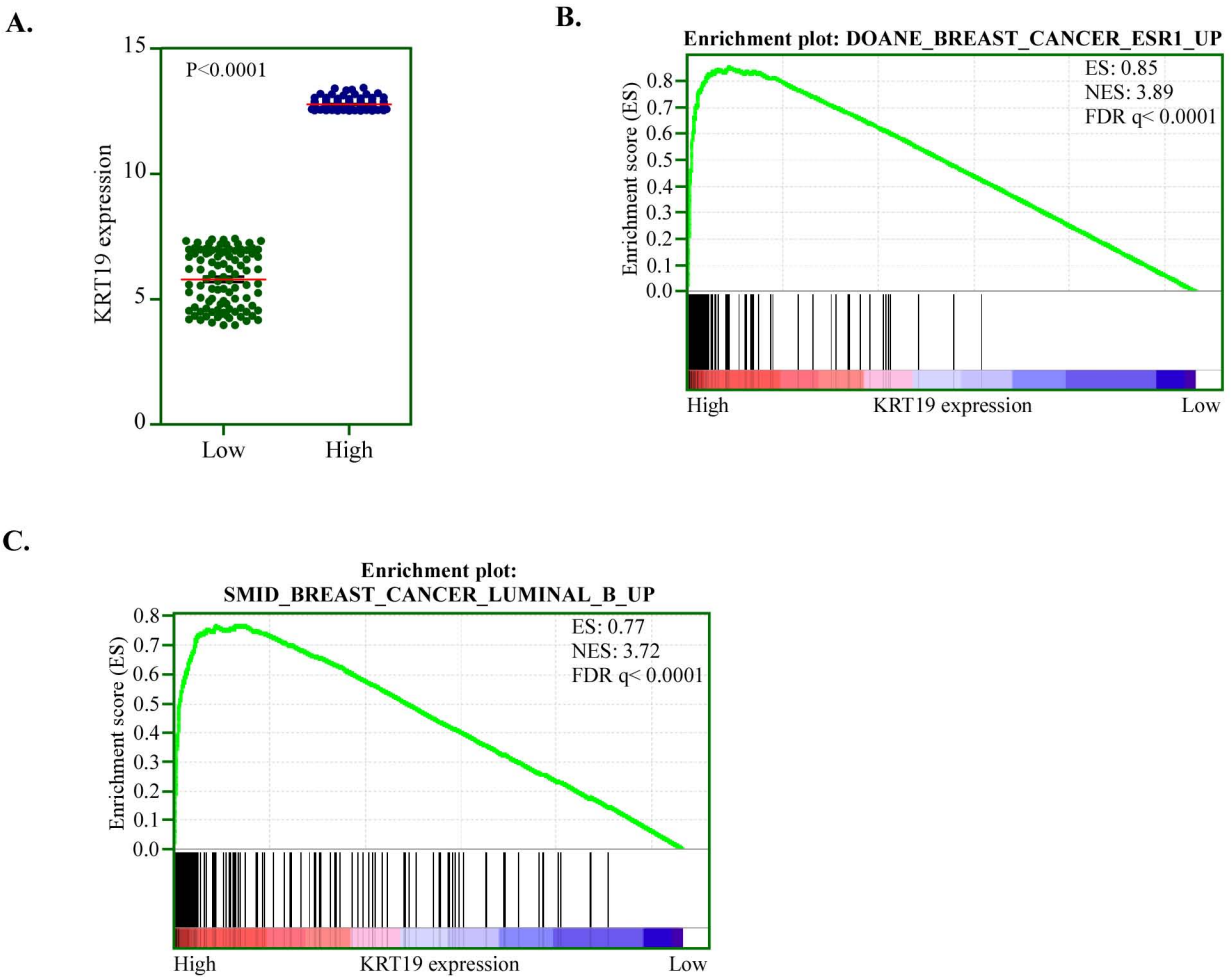
A.
P<0.0001
KRT19 expression
15
10
5
0
Low
High
B.
Enrichment plot: DOANE_BREAST_CANCER_ESR1_UP
ES: 0.85
NES: 3.89
FDR q< 0.0001
Enrichment score (ES)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
High
KRT19 expression
Low
C.
Enrichment plot:
SMID_BREAST_CANCER_LUMINAL_B_UP
ES: 0.77
NES: 3.72
FDR q< 0.0001
Enrichment score (ES)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
High
KRT19 expression
Low

Figure 3

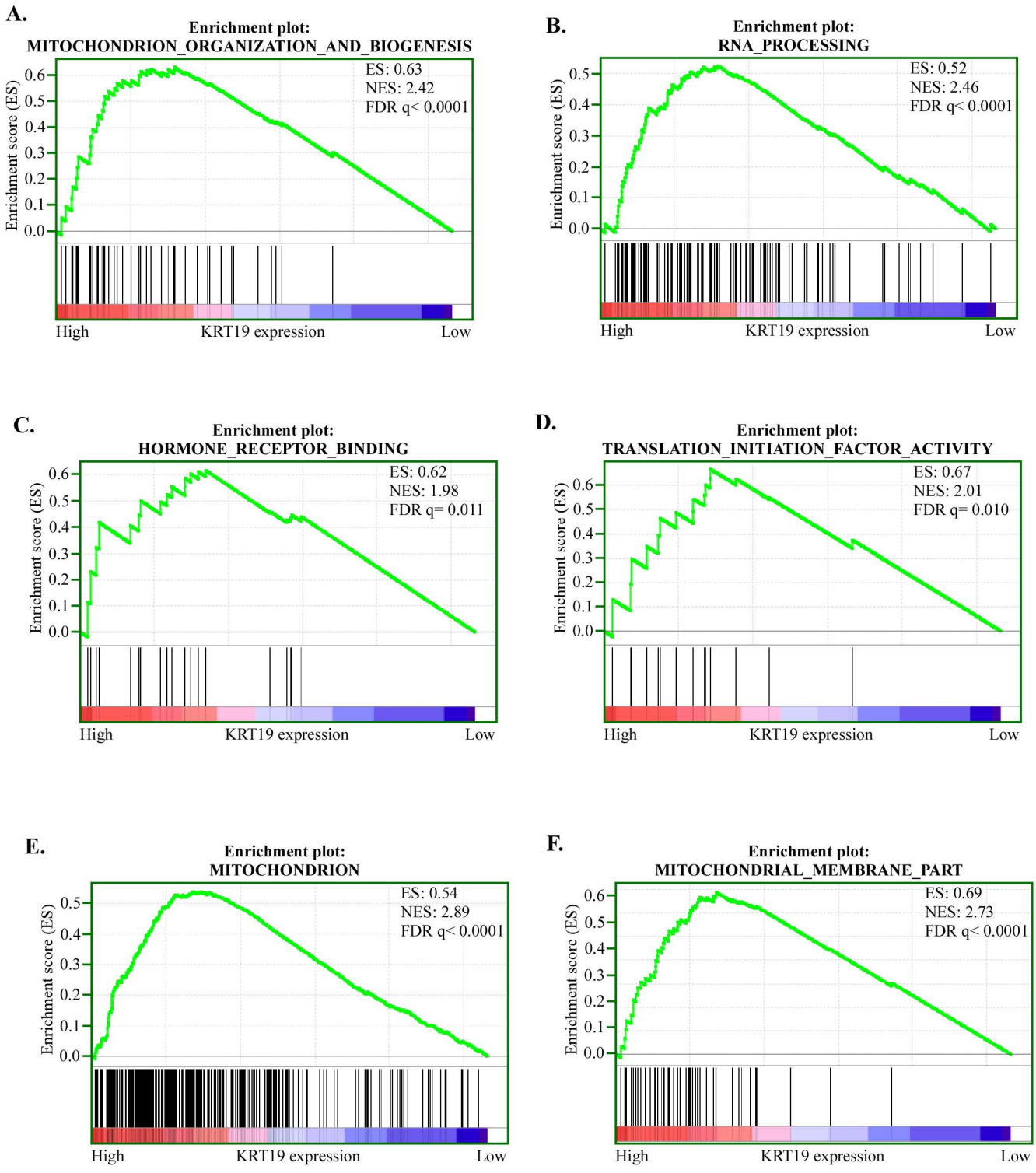
A.
Enrichment plot:
MITOCHONDRION_ORGANIZATION_AND_BIOGENESIS
ES: 0.63
NES: 2.42
FDR q< 0.0001
Enrichment score (ES)
High
KRT19 expression
Low
B.
Enrichment plot:
RNA_PROCESSING
ES: 0.52
NES: 2.46
FDR q< 0.0001
C.
Enrichment plot:
HORMONE_RECEPTOR_BINDING
ES: 0.62
NES: 1.98
FDR q= 0.011
D.
Enrichment plot:
TRANSLATION_INITIATION_FACTOR_ACTIVITY
ES: 0.67
NES: 2.01
FDR q= 0.010
E.
Enrichment plot:
MITOCHONDRION
ES: 0.54
NES: 2.89
FDR q< 0.0001
F.
Enrichment plot:
MITOCHONDRIAL_MEMBRANE_PART
ES: 0.69
NES: 2.73
FDR q< 0.0001

Figure 4

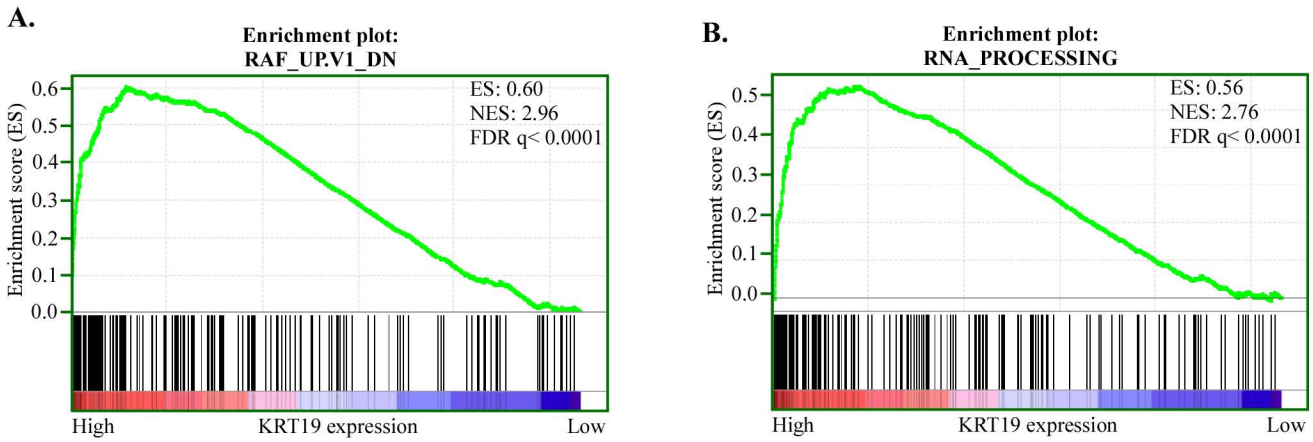
A.
Enrichment plot:
RAF_UP.V1_DN
ES: 0.60
NES: 2.96
FDR q< 0.0001
Enrichment score (ES)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
High
KRT19 expression
Low
B.
Enrichment plot:
RNA_PROCESSING
ES: 0.56
NES: 2.76
FDR q< 0.0001
Enrichment score (ES)
0.5
0.4
0.3
0.2
0.1
0.0
High
KRT19 expression
Low

Figure 5

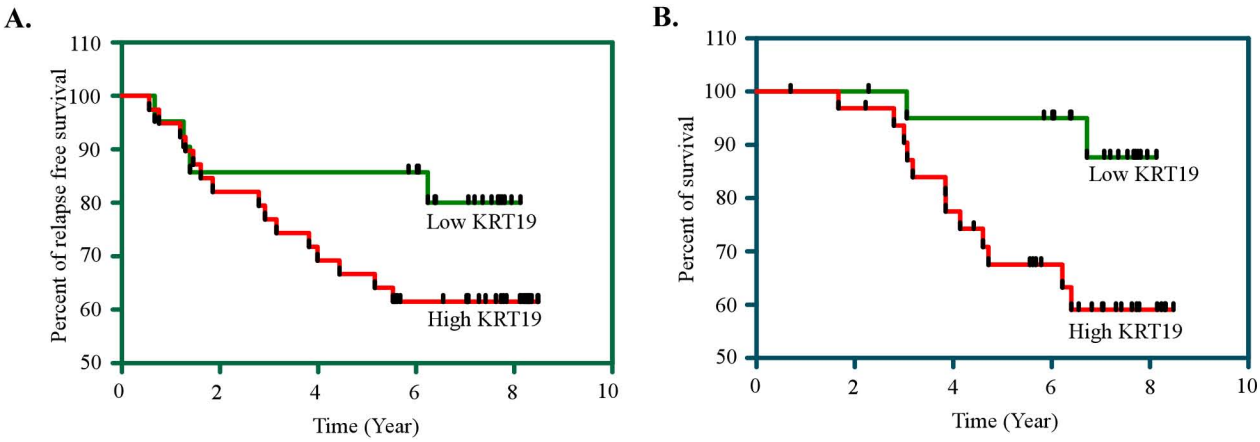
A.
Percent of relapse free survival
110
100
90
80
70
60
50
0
2
4
6
8
10
Time (Year)
Low KRT19
High KRT19
B.
Percent of survival
110
100
90
80
70
60
50
0
2
4
6
8
10
Time (Year)
Low KRT19
High KRT19

Figure 6