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ORIGINAL RESEARCH

SYK expression level distinguishes control from *BRCA1*-mutated lymphocytes

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Background: About 5%–10% of breast cancer and 10%–15% of ovarian cancer are hereditary. *BRCA1* and *BRCA2* are the most common germline mutations found in both inherited breast and ovarian cancers. Once these mutations are identified and classified, a course of action to reduce the risk of developing either ovarian or breast cancer – including surveillance and surgery – is carried out.

Purpose: The purpose of the current research is to characterize the gene expression differences between healthy cells harboring a mutation in *BRCA1*/2 genes and normal cells. This will allow detection of candidate genes and help identify women who carry functional *BRCA1*/2 mutations, which cannot always be detected by the available sequencing methods, for example, carriers of mutations found in regulatory sequences of the genes.

Materials and methods: Our cohort consisted of 50 healthy women, of whom 24 were individuals with *BRCA1* or *BRCA2* heterozygous mutations and 26 were non-carrier controls. RNA purified from non-irradiated lymphocytes of nine *BRCA1*/2 mutation carriers versus four control mutation-negative individuals was utilized for RNA-Seq analysis. The selected RNA-Seq transcripts were validated, and the levels of spleen tyrosine kinase (*SYK*) mRNA were measured by using real-time quantitative polymerase chain reaction.

Results: Differences in gene expression were found when comparing untreated lymphocytes of *BRCA1/2* mutation carriers and controls. Among others, the *SYK* gene was identified as being differently expressed for *BRCA1*/2 mutation carriers. The expression level of *SYK* was significantly higher in untreated healthy lymphocytes of *BRCA1* heterozygote carriers compared with controls, regardless of irradiation. In contrast to normal tissues, in cancerous breast tissues, the expression levels of the *BRCA1* and *SYK* genes were not intercorrelated.

Conclusion: Collectively, our observations demonstrate that *SYK* may prove to be a good candidate for better diagnosis, treatment, and prevention of *BRCA1* mutation-associated breast cancer.

Keywords: RNA-Seq, breast cancer susceptibility, peripheral blood

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Introduction

The presence of mutations in *BRCA1* or *BRCA2* genes increases a woman's susceptibility to develop breast and ovarian cancers by the age of 70, with the incidence ranging between 47% and 66%, and 40% and 57%, respectively.[1,2] Additionally, women with a *BRCA* mutation have an elevated risk of developing other malignancies, such as gastrointestinal cancers (e.g., gall bladder, bile duct, colon, stomach, pancreas) and melanoma, and when this mutation appears in males, it increases the risk for breast and prostate cancers.[3,4]

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The BRCA1 and BRCA2 proteins are involved in different cellular processes, including homologous recombination of DNA repair,[5,6] ubiquitination, chromosomal segregation,[7] cell cycle arrest, apoptosis, and gene transcription. In addition, they are required for the S phase and G2/M checkpoint arrest in response to DNA damage.[8–10] Abnormal *BRCA1* and *BRCA2* expression interferes with routine cell cycle events such as cell division, death, or life span. Therefore, these genes are recognized as "gatekeeper" genes, whose mutation stimulates the progression of cancer.[11]

Although the *BRCA1* and *BRCA2* genes were identified a long time ago, their specific biological mechanisms and the way disruptions of their functions promote breast and ovarian carcinogenesis remain unclear. According to Knudson's widely accepted "two-hit" hypothesis,[12] inheriting one de novo germline copy of a mutated *BRCA1* or *BRCA2* gene (i.e., the so-called first hit) is insufficient to enable the development of breast cancer and other hereditary cancer syndromes. The acquisition of a "second hit" to the remaining "healthy" copy of the gene is required for cancer development. This mutation can occur somatically, leading to the loss of both copies of the normal tumor suppressor gene. Notwithstanding, several studies support the idea that haploinsufficiency itself can interrupt normal cell function by contributing to genome instability, which leads to additional cancer driver mutations. For example, mice carrying a heterozygous mutation in the *BRCA1* gene have shortened life spans and are more susceptible to ovarian cancer following ionizing irradiation without losing the other *BRCA1* allele.[13] Such findings suggest that women carrying *BRCA1* mutations may be more susceptible to ovarian tumor formation after irradiation than non-mutation carriers.

Different cell types, such as normal fibroblasts[14] and ovarian and breast epithelial cells[15] from heterozygous *BRCA1* mutation carriers, display a different gene expression profile than controls in response to DNA damage, again suggesting that *BRCA* heterozygosity itself contributes to breast cancer initiation. Moreover, it was found that losing a single allele of *BRCA1* contributes specifically to alteration in the expression of genes having a role in cellular differentiation.[16] This finding supports the hypothesis that single copy loss of *BRCA1* may cause variations in cell differentiation and, eventually, causes cells to undergo malignant processes.[16] Our previous study demonstrated the differences in gene expression in lymphocytes subject to ionizing radiation derived from *BRCA* mutation carriers as compared with controls, confirming a measurable heterozygous effect.[17] Finally, it has been shown using fluorescence lifetime (FLT) imaging microscopy, a method used to differentiate between distinct cell populations, that lymphocytes derived from control or *BRCA1* mutation carriers differ in their FLT values from lymphocytes derived from *BRCA2* mutation carrier individuals. This study demonstrates that *BRCA1* and *BRCA2* heterozygous lymphocytes have innate differences independent of irradiation. In addition, it suggests that these heterozygous mutations can lead to changes in the physical properties of the cells themselves, such as pH, temperature, viscosity, and oxygen concentration.[18]

In the current study, we wished to extend our previous research and examine whether there is a difference in gene expression in untreated cells from *BRCA* carriers and controls. The spleen tyrosine kinase (*SYK*) gene was identified as differently expressed in *BRCA1* mutation carriers. The results of this study will allow for a more accurate characterization of intrinsic differences between healthy cells harboring a mutation in *BRCA1/2* genes and normal cells. Moreover, our observations demonstrate that *SYK* could prove to be a good candidate for more effective diagnosis, treatment, and prevention of *BRCA1* mutation-associated breast cancer.

Materials and methods

Sample information

Our cohort consisted of 50 healthy women aged between 25 and 50 years, of whom 24 were individuals with no personal history of cancer but with *BRCA1* or *BRCA2* heterozygous mutations and 26 women had no *BRCA* mutations. Note that some of the subjects in the various analyses (RNA-Seq and real-time quantitative polymerase chain reaction [qPCR]) in this study overlap. The mutation carriers were diagnosed in the *BRCA1* and *BRCA2* predictive testing program of the Oncogenetic Clinic of Hadassah University Medical Center (Jerusalem, Israel).

The women in the study who served as controls needed to fulfill two conditions. The first one was the absence of any personal or familial history of breast or ovarian cancer in the participants. Second, they needed to be non-carriers of known *BRCA1* or *BRCA2* mutations.

The local Ethics Committee of Hadassah Medical Center approved the study protocol, and written informed consent was obtained from all participating individuals before inclusion in the study.

Lymphocyte extraction and preparation

Lymphocyte extraction and treatment were conducted as previously described.[17] In summary, primary lymphocyte cells were obtained from peripheral blood using LymphoPrep

(Sigma) and short-term cultured for 6 days in RPMI-1640 with L-glutamine (Biological Industries), supplemented with interleukin-2, 15% fetal calf serum, 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and 1% penicillin/streptomycin. One day following lymphocyte extraction, half of each sample was γ-irradiated with 8 Gy at a high-dose rate (0.86 Gy/min, Orthovoltage X-ray machine), as previously described.[19] Although this dose exceeds the portion used in classical radiotherapy or screening radiation, the DNA damage it causes presumably mimics the additive effect of repeated radiation exposure, which usually occurs in the clinic.[9] Additionally, we chose this dose based on our previous study showing differences in gene expression in *BRCA* mutation carriers after ionizing radiation.[17]

Gene expression analysis

Poly(A)-selected RNA was sequenced using the Illumina TruSeq protocol on the HiSeq 2500 sequencing machine. Quality control checks on the raw sequence data were carried out using the FastQC tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Then, the Trim Galore! tool (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/), which is based on cutadapt,[20] was used for adapter trimming and for removing low-quality bases from the ends of reads. Clean reads were mapped to the human genome (hg38) using tophat2.[21] Next, the number of reads mapping each human gene (as annotated in Ensembl release 77) was counted using the "union" mode of HTseq-count script.[22] Differential expression analysis was performed using the edgeR and Limma packages from the Bioconductor framework.[23] Briefly, features with <1 read per million in at least five samples were removed. The remaining gene counts were normalized using the trimmed mean of M values method, followed by voom transformation.[24,25] Linear models, as implemented by the Limma package,[23] were used to find differentially expressed genes. Since FDR application was too stringent for this data set, genes with a *P*-value <0.001 were considered as differentially expressed. Gene set enrichment and pathway analysis were done using GeneAnalytics.[26]

RNA extraction and real-time qPCR

Total RNA was extracted from primary lymphocyte cells using a Direct-zol™ RNA MiniPrep kit (Zymo Research), according to the protocol recommended by the manufacturer. Complementary DNA was obtained by reverse transcription of 850 ng of total RNA in a final reaction volume of 20 µL containing 4 µL qScript Reaction Mix and 1 µL qScript Reverse Transcriptase (Quantabio). Quantitative real-time PCR assays containing the primers (Table S1) and probe mix for *SYK*, *TSPAN18*, *DPEPT*, and *OTUB2* were purchased from Biosearch Technologies and utilized according to the manufacturer's instructions. PCR was carried out in a final reaction volume of 10 µL containing 20 ng of complementary DNA template, 5 µL of PerfeCTa SYBR Green FastMix, ROX (Quantabio), and 1 µL of primers mix. All reactions were run in triplicate, and the housekeeping genes, *GAPDH* (Figure S1) and *beta-actin* (Figure S2), were amplified in a parallel reaction for normalization.

Analysis of publicly available ICGC and GTEx expression data

The expression values of *BRCA1*, *BRCA2*, and *SYK* genes across the breast tissues taken from healthy donors and patients with breast cancer (available from the GTEx[27] and ICGC[28] databases, respectively) were visualized using the UCSC Xena genome browser.[29] In each database, samples were divided into two groups based on the expression of *BRCA1* or *BRCA2* genes using the median value as the cutoff point. The chart view option in Xena was used to view the distribution of *SYK* expression across the two groups and to calculate the Welch's *t*-test. Mean centered values of the normalized *SYK* expression were downloaded and box plots were generated using R language.

Results

Characterizing the mRNA expression profile in *BRCA1/2* mutation carriers versus non-carrier controls

In a previous study, we tested whether *BRCA1* or *BRCA2* mutation carriers could be distinguished from control mutation-negative individuals based on gene expression changes triggered by the activation of *BRCA1*/2 genes following irradiation.[17] As opposed to the previous study, herein, we tested for differences in basal gene expression in these groups using non-irradiated lymphocytes. We used an RNA-Seq analysis on lymphocyte-derived RNA to evaluate the gene expression in *BRCA1* and *BRCA2* heterozygous mutants and mutation-free individuals. The mutation types identified in carriers used in this study are summarized in Table 1. Notably, all the mutations are known to be functional and contribute to the risk of developing breast cancer. When comparing the control group to *BRCA1*/2 together, 30 genes were significantly differentially expressed (*P*<0.001; Figure 1A; Table S2). Furthermore, when we did a separate statistical analysis on *BRCA1* and *BRCA2,* as compared

Table 1 Mutation data of patients enrolled in the study

Mutation	Number of individuals
BRCA1-185del AG	8
BRCA1-5382 ins C	1
BRCA1-Q356R	1
BRCA1-p.P1812A	1
BRCA2-6174delT	10
BRCA2-c.6024dupG	1
BRCA2-R2336p	1
BRCA2-IVS2+1G>A	1

with the control group, we were able to identify 203 genes in *BRCA1* and 29 genes in *BRCA2* that were differentially expressed (Figure 1B and 1C; Table S2).

Differently expressed genes that may predict *BRCA1* status, but not *BRCA2*, indicate defects in DNA repair

To further understand the underlying biology leading to the classification schemes (Figure 1), we used the GeneAnalytics application[26] to enable us to examine the networks formed from the differentially regulated genes in *BRCA1* mutation carriers.

Genes differentially expressed in *BRCA1* mutation carriers versus control (Figure 1B) are involved in tumorigenic-related pathways (Figure 2). Most of these genes (122) are downregulated and are involved in critical cellular functions, including cell division, DNA replication, and DNA repair (Figure 2A). Analysis of 81 upregulated genes revealed over-representation of genes involved in the T-cell receptor signaling, immune system, and B-cell receptor signaling pathway (Figure 2B). Surprisingly, although the *BRCA2* gene has been implicated in a diverse array of cellular functions, including DNA repair, differential gene expression among *BRCA2* mutation carriers and controls revealed only 29 differently expressed genes (Figure 1C), none of which indicated any significant defects in DNA repair or other cellular signaling (data not shown).

Validation of selected RNA-Seq transcripts by real-time qPCR

To ensure technical reproducibility and to validate our observation, we conducted a real-time qPCR analysis of those transcripts identified as significantly differently expressed for *BRCA1/2* mutation carriers, and their expression levels in samples were relatively high. Lymphocytes were obtained from 12 control mutation-negative individuals and 14 *BRCA1/2* mutation carriers (Table 1). RNA was prepared

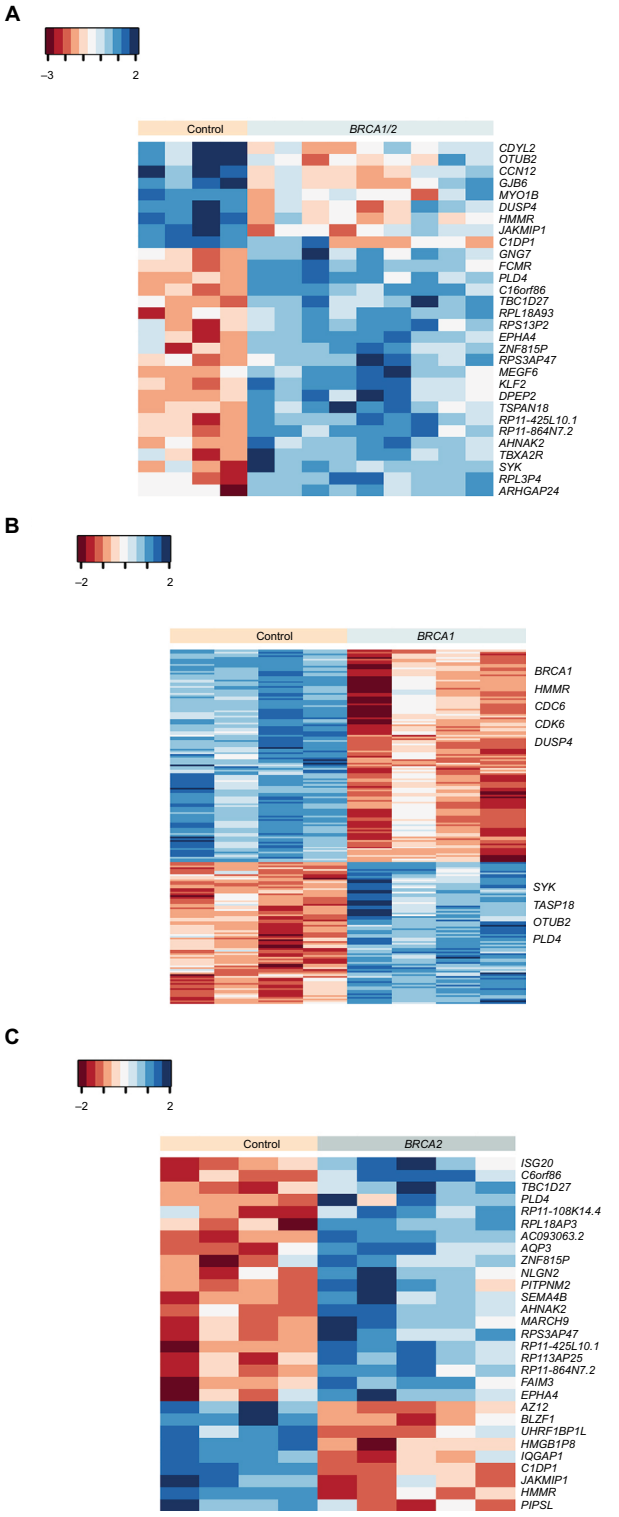


Figure 1 Basal gene expression profile in *BRCA1* and/or *BRCA2* mutation carriers versus non-carriers.

Notes: Heat map of hierarchical cluster analysis showing 30 DE genes ($P<0.001$, fold change ≥2) between *BRCA1/2* mutation carriers and control samples (n=4–5/group) **(A)**, 203 DE genes ($P<0.001$) between *BRCA1* mutation carriers and controls **(B)**, and 29 DE genes ($P<0.001$) between *BRCA2* carriers versus non-carriers **(C)**. Red indicates a decrease in gene expression, while blue indicates an increase. Euclidean distance was used as the distance method and complete linkage as the agglomeration method.

Abbreviation: DE, differentially expressed.

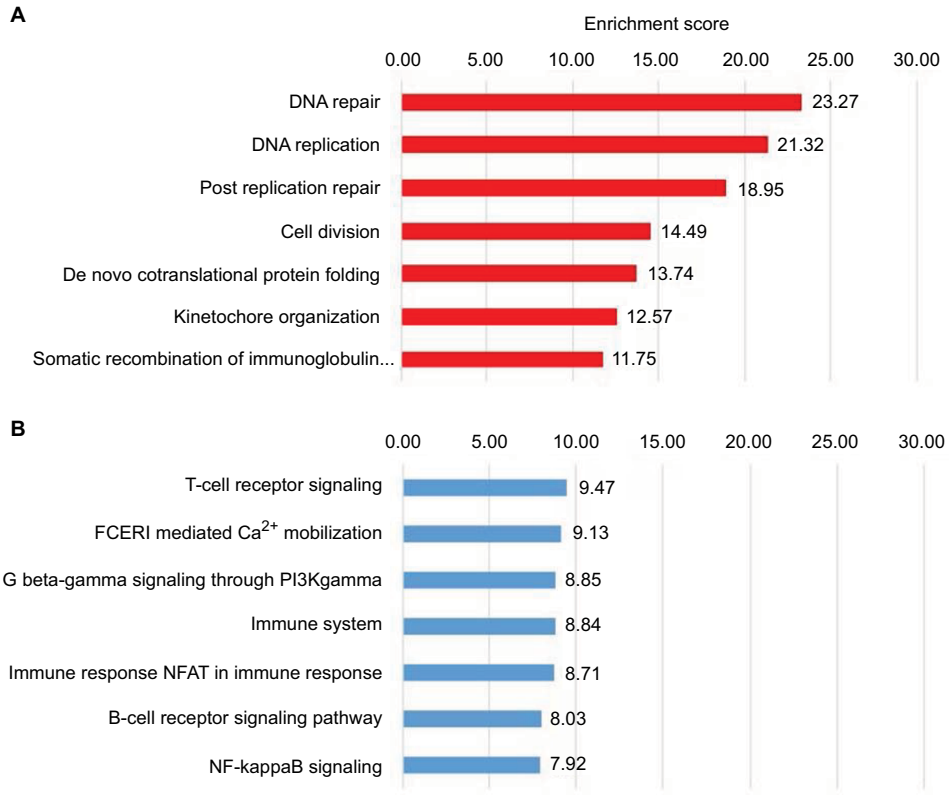


Figure 2 Analysis of genes predicting *BRCA1* status.
Notes: GeneAnalytics analysis of the 203 genes differentially regulated in cells derived from *BRCA1* mutation carriers in the RNA-Seq data. One hundred and twenty-two downregulated **(A)** and 81 upregulated **(B)** genes were subjected to enrichment analysis of Gene Ontologies–Biological Processes.
Abbreviations: NFAT, nuclear factors of activated T-cells; FCERI, Fc epsilon receptor.

and subjected to the SYBR Green assay for the indicated target genes. As presented in Figure 3A, although the expression of *TSPAN18*, *SYK,* and *DPEPT* showed consistency between RNA-Seq and real-time qPCR-based approaches in terms of overall average expression values across multiple samples, these differences were statistically significant only for *TSPAN18*. No variations in *OTUB2* gene expression were observed among the different groups. Comparing the control mutation-negative individuals to *BRCA1* mutation carriers only (Figures 3B and 4A) revealed significant differences in the expression of *TSPAN18* and *SYK*.

SYK expression levels may distinguish control from *BRCA1*-mutated lymphocytes

Oxidative stress of B-cell precursor acute lymphoblastic leukemia cells following ionizing radiation leads to the activation of PLK1 protein kinase, which then turns on the SYK protein.[30,31] Since accumulating evidence suggests that BRCA1 and BRCA2 regulate ionizing radiation-induced oxidative stress,[32] we hypothesized that the mutation status of *BRCA1* and *BRCA2* genes directly affects the *SYK* expression profile following irradiation. We tested this hypothesis by examining the levels of *SYK* mRNA in control mutation-negative individuals and *BRCA1* or *BRCA2* mutation carriers using irradiated human lymphocytes.

For this purpose, primary lymphocytes obtained from peripheral blood were γ-irradiated with 8 Gy. RNA was extracted from the lymphocytes of *BRCA1*/2 mutation carriers and controls 1 hour following irradiation (10 and 11 subjects per group, respectively) and from lymphocytes of *BRCA1*/2 mutation carriers and controls, 6 days following irradiation (17 and 10 subjects per group, respectively), and a real-time qPCR analysis was conducted.

Our results demonstrate that, 6 days post-ionizing irradiation, the expression of *SYK* was, indeed, significantly upregulated in the lymphocytes of *BRCA1* heterozygote carriers, as compared with controls. In contrast, 1 hour following irradiation, there were no differences in the expression levels of the gene among the different groups (Figures 4B-C and S2). Notably, even without irradiation, the expression of *SYK* was significantly upregulated in short-term cultured lymphocytes of *BRCA1* mutation carriers, as compared with *BRCA2* (Figures 3B and 4A).

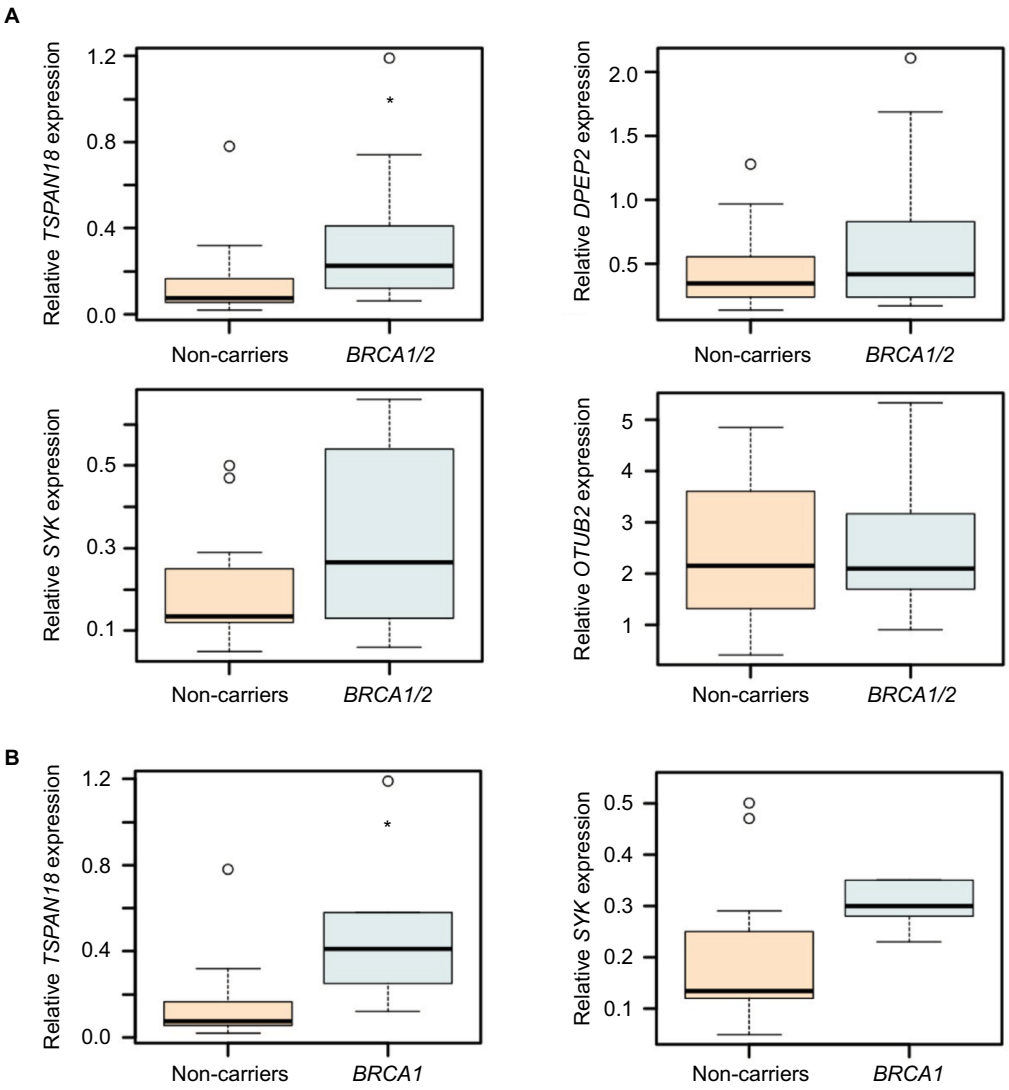


Figure 3 Predictive transcripts for *BRCA1/2* mutation carriers.
Notes: Distribution of *OTUB2, TSPAN18*, *SYK*, and *DPEPT* expression levels in lymphocytes of 12 control mutation-negative individuals and 14 *BRCA1/2* mutation carriers, as determined by real-time qPCR **(A)**. Distribution of *TSPAN18* and *SYK* expression levels across lymphocytes of mutation-negative individuals (n=12) and *BRCA1* mutation carriers (n=5) **(B)**. *GAPDH* was used for normalization (Figure S1). *$P<0.05$, calculated using Student's *t*-test.
Abbreviations: qPCR, quantitative polymerase chain reaction; SYK, spleen tyrosine kinase.

Expression pattern of *BRCA1/2* and *SYK* in patients with breast cancer

SYK is also expressed in the breast tissue.[33] Considerable evidence demonstrates that SYK functions as a tumor suppressor in this tissue. Taking into account our results with lymphocytes, which provide evidence for a link between *SYK* expression and heterozygous *BRCA1/2* mutations, and the known fact that SYK plays a critical role in breast cancer, an effort was made to identify correlations between the expression pattern of *BRCA1/2* and *SYK* genes in breast cancer.

To accomplish this aim, we first analyzed the published GTEx dataset to gain a view on *BRCA1*, *BRCA2*, and *SYK* gene expression in normal breast tissues. As shown in Figure 5A and 5B, we found that samples having low *BRCA1* or *BRCA2* expression exhibit lower *SYK* expression and vice versa. Next, we analyzed the ICGC datasets of breast tumors. This analysis showed that, in contrast to normal tissues, the expression levels of the *BRCA1* and *SYK* genes were not intercorrelated in breast samples from patients with ductal or lobular breast cancer (Figure 5C and 5D). Conversely, the expression levels of *BRCA2* in these tissues were positively correlated with those of *SYK*, which was similar to the expression pattern of these genes in healthy breast tissues (Figure 5C and 5D). Taken together, our observations suggest that determining the expression levels of both *BRCA1* and *SYK* in the same tumor may be clinically significant.

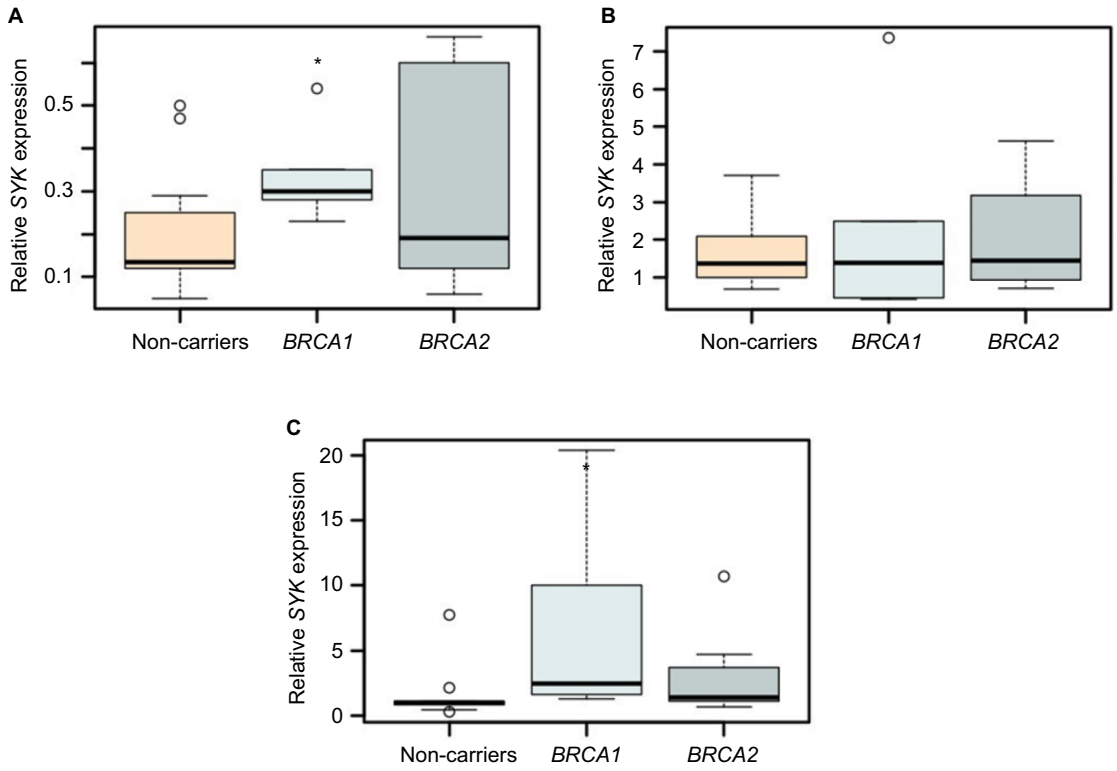


Figure 4 *SYK* expression levels may distinguish control from *BRCA1*-mutated lymphocytes.
Notes: The box plots represent the expression of *SYK* in lymphocytes of *BRCA1*/2 mutation carriers and controls without irradiation **(A)** and at 1 hour **(B)** and 6 days **(C)** following irradiation, as determined by real-time qPCR. *GAPDH* was used for normalization. (n=4–12/group). For all experiments, **P*<0.05, calculated using Student's *t*-test.
Abbreviations: qPCR, quantitative polymerase chain reaction; SYK, spleen tyrosine kinase.

Discussion

Our RNA-Seq analysis demonstrates that when a group of *BRCA1* and *BRCA2* mutation carriers are compared to *BRCA* mutation-free controls, genes are differentially expressed (Figure 1). However, larger and more significant differences were found in the comparison between *BRCA1* mutation carriers and control groups, and these include many functionally crucial genes, such as *SLF1*, *MSH2*, *MSH6*, *CLSPN*, *TOPBP1*, *UBE2A*, *BRCA1*, and others involved in DNA repair and replication pathways. Previously, Vuillaume et al[34] attempted to identify *BRCA1* mutation carriers based on the gene expression pattern in peripheral blood lymphocytes. Unlike our results, they found no significant difference in gene expression patterns between *BRCA1* mutation carriers and controls. We assume that we were able to distinguish *BRCA1* mutation carriers from non-carriers because we used RNA-Seq technology, which has superior benefits over microarray analyses in transcriptome profiling.[35] Furthermore, in our study, short-term lymphocyte cultures were established for 6 days after obtaining the cells, instead of immediate RNA isolation. By doing so, lymphocyte populations became less heterogeneous between samples obtained from different individuals, and the results were affected mainly by *BRCA* carrier status rather than other factors associated with the donor of the lymphocytes.

It is already known that *BRCA1* and *BRCA2* have distinct functions in DNA repair following ionizing radiation-induced DNA damage and are key players at different stages of homologous recombination, which provides a mechanism to precisely repair damage.[36] Therefore, it is not surprising that, after irradiation, the gene expression profile differs, based on which of the two *BRCA* genes is mutated.[17] However, here, we demonstrate, for the first time, that even without irradiation, *BRCA1*- and *BRCA2*-mutated cells differ in their gene expression profiles. These results corroborate our previous findings, in which cells carrying the *BRCA1* mutation showed higher FLT values than those carrying *BRCA2,* suggesting an intrinsic difference between the carriers of the two mutations.[18]

Overall, greater differences in gene expression, in terms of the number and type of genes, were observed when comparing *BRCA1*, but not *BRCA2,* mutation carriers to the control group. These differences may be explained because the *BRCA1* gene itself is downregulated in *BRCA1* mutation carriers compared with controls, while the *BRCA2* gene is not altered in *BRCA2* mutation carriers (Figure 1B).

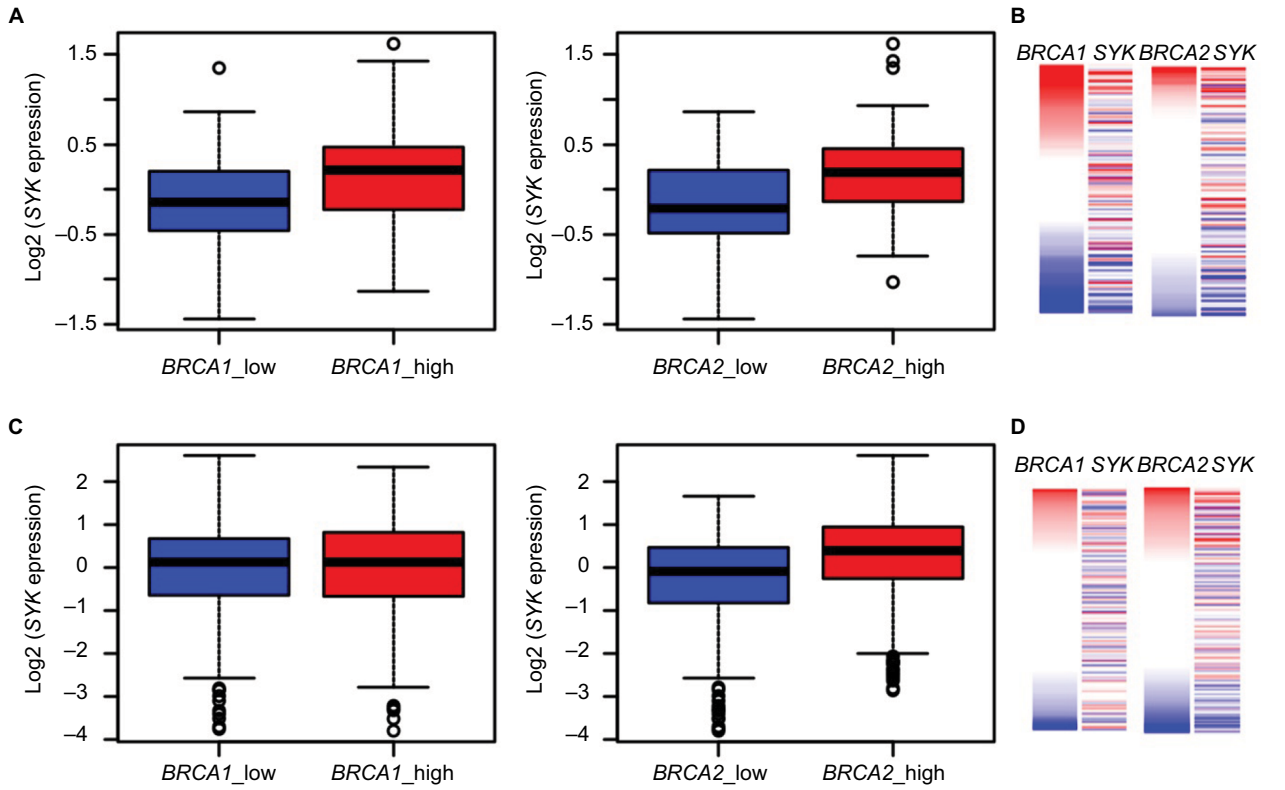


Figure 5 Association between *BRCA2* and *SYK* expression in normal and cancerous breast tissues.
Notes: Healthy breast tissues (n=214) from the GTEx database **(A, B)** and breast cancer samples (n=943) from the ICGC database **(C, D)** were divided into two groups based on the expression of *BRCA1* **(A, C)** and *BRCA2* **(B, D)** genes, using the median as the cutoff point. The box plots display the expression distribution of *SYK* across these groups. Higher *SYK* expression was detected in healthy breast tissues exhibiting an increased level of *BRCA1* **(A)** $P=2.391\times10^{-6}$ and *BRCA2* expression **(B)** $P=5.122\times10^{-7}$. In cancerous breast tissues, this association is preserved for *BRCA2* **(D)** $P=6.198\times10^{-15}$, but not for *BRCA1* **(C)** $P=1.000$.
Abbreviation: SYK, spleen tyrosine kinase.

The available methods for identifying *BRCA1* and *BRCA2* mutation carriers are based on sequencing approaches and do not detect all functional mutations (e.g., carriers with regulatory mutations, which are found in regulatory sequences of the genes). Previously, we suggested a functional diagnostic tool for identifying *BRCA* mutation carriers by using fresh lymphocytes.[17] However, this approach cannot be easily applied since it requires irradiation of fresh lymphocytes by an X-ray machine, which is not available in most medical centers. Here, we demonstrate the potential of using real-time qPCR analysis in setting up a functional screening tool for *BRCA1* mutation carriers in untreated accessible blood lymphocytes. Further studies in larger cohorts are needed to identify a set of specific genes to be used by this tool and verify its clinical efficacy. As presented in Figure 3, the *SYK* gene is a major candidate to be used by this tool. SYK is primarily expressed in hematopoietic cells. In lymphoid cells, this protein tyrosine kinase regulates several signaling pathways and is of great importance in B-cell receptor signaling, functioning as the main downstream effector protein. It has been shown that ionizing radiation in B-cell precursor acute lymphoblastic leukemia cells leads to oxidative stress and the activation of PLK1 protein kinase, which induces SYK enzymatic activity.[30,31] In addition to our findings showing that *SYK* expression levels can distinguish controls from *BRCA1*-mutated lymphocytes, the study of gene activity in *BRCA* carriers may have other clinical implications, especially as SYK functions as a tumor suppressor.[33,37] As shown in Figure 4, *SYK* expression level was significantly higher in lymphocytes of *BRCA1* heterozygote carriers, as compared with controls, regardless of irradiation. Six days following the ionizing irradiation, the differences were even greater and more significant. A similar phenomenon can also be seen in normal breast tissue where samples with low *BRCA1* expression exhibit lower *SYK* expression and vice versa. In contrast, in cancerous breast tissues, the situation is different; no link was found between the expression levels of the two genes, *BRCA1* and *SYK* (Figure 5). We assume that, in healthy lymphocytes, the oxidative stress due to a mutation in *BRCA1* increases, especially after ionizing irradiation, but a corresponding increased level of *SYK* expression and its suppressor function help maintain normal cell activity. Furthermore, in normal breast tissue, since both

SYK and BRCA1 are tumor suppressors, we are not surprised that their expression pattern is similar. However, in cancerous tissues, such as breast tumors, even though *BRCA1* is lowly expressed, the lack of corresponding increase in *SYK* expression supports the disruption of normal cellular activity, thus contributing to tumor formation and progression. In addition to research indicating a clear link between mutations in *BRCA1* and the development of breast cancer,[2] this hypothesis is supported by various studies demonstrating that *SYK* is expressed in normal human breast tissue, and in benign breast lesions and low-tumorigenic breast cancer cell lines.[37,40] However, in the case of invasive breast carcinoma tissues and cell lines, the expression of *SYK* mRNA and protein is low compared to normal expression. This reduced *SYK* expression increases the risk of distant metastasis and also causes a poorer prognosis in a few tumor types, including breast carcinoma, hepatocellular carcinoma, oral squamous cell carcinoma, and melanoma.[38]

From a clinical perspective, the prognostic usefulness of reduced *SYK* levels in *BRCA1* mutation and/or lowly expressed *BRCA1*-associated breast cancer should be investigated.

In normal breast tissue, when the expression levels of *BRCA2* are low, the expression levels of *SYK* are correspondingly low and vice versa. Unlike *BRCA1*, in breast tumors, *BRCA2* and *SYK* behave similarly – when the expression level of one is high, the other one is highly expressed and vice versa (Figure 5). These results again support the notion that both proteins, *BRCA1* and *BRCA2*, have distinct functions in DNA repair and cell cycle progression.

Finally, poly(ADP-ribose) polymerase inhibitors represent a promising strategy for treating BRCA-associated cancers harboring specific DNA repair defects.[39] However, we still need to investigate and understand the proper use of these agents. Some of the challenges that we face include identifying predictive biomarkers of response, which may be used for selecting a subset of patients who would maximally benefit from treatment with this novel agent; determining optimal dose and schedule; and establishing efficacy as a single agent or in combination with other chemotherapeutics. Since *SYK* expression increases due to oxidative stress and DNA damage, we assume that patients expressing high levels of *SYK* mRNA will respond better to treatment with poly(ADP-ribose) polymerase inhibitors.

Conclusion

Collectively, our observations demonstrate that *SYK* may be a good candidate for better diagnosis, treatment, and prevention of *BRCA1* mutation-associated breast cancer. However, more detailed studies are needed to gain a clearer picture regarding the role of *SYK* in the pathogenesis of *BRCA* mutation-associated tumors.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Stoppa-Lyonnet D. The biological effects and clinical implications of BRCA mutations: where do we go from here? *Eur J Hum Genet*. 2016;24(Suppl 1):S3–S9.
2. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. *J Clin Oncol*. 2007;25(11):1329–1333.
3. Thompson D, Easton D. The genetic epidemiology of breast cancer genes. *J Mammary Gland Biol Neoplasia*. 2004;9(3):221–236.
4. Mersch J, Jackson MA, Park M, et al. Cancers associated with BRCA1 and BRCA2 mutations other than breast and ovarian. *Cancer*. 2015;121(2):269–275.
5. Moynahan ME, Chiu JW, Koller BH, Jasin M. Brca1 controls homology-directed DNA repair. *Mol Cell*. 1999;4(4):511–518.
6. Tutt A, Bertwistle D, Valentine J, et al. Mutation in Brca2 stimulates error-prone homology-directed repair of DNA double-strand breaks occurring between repeated sequences. *EMBO J*. 2001;20(17):4704–4716.
7. Starita LM, Machida Y, Sankaran S, et al. BRCA1-dependent ubiquitination of gamma-tubulin regulates centrosome number. *Mol Cell Biol*. 2004;24(19):8457–8466.
8. Kote-Jarai Z, Matthews L, Osorio A, et al. Accurate prediction of BRCA1 and BRCA2 heterozygous genotype using expression profiling after induced DNA damage. *Clin Cancer Res*. 2006;12(13):3896–3901.
9. Kote-Jarai Z, Salmon A, Mengitsu T, et al. Increased level of chromosomal damage after irradiation of lymphocytes from BRCA1 mutation carriers. *Br J Cancer*. 2006;94(2):308–310.
10. Ernestos B, Nikolaos P, Koulis G, et al. Increased chromosomal radiosensitivity in women carrying BRCA1/BRCA2 mutations assessed with the G2 assay. *Int J Radiat Oncol Biol Phys*. 2010;76(4):1199–1205.
11. Boulton SJ. Cellular functions of the BRCA tumour-suppressor proteins. *Biochem Soc Trans*. 2006;34(Pt 5):633–645.
12. Knudson AG. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA*. 1971;68(4):820–823.
13. Jeng YM, Cai-Ng S, Li A, et al. Brca1 heterozygous mice have shortened life span and are prone to ovarian tumorigenesis with haploinsufficiency upon ionizing irradiation. *Oncogene*. 2007;26(42):6160–6166.
14. Kote-Jarai Z, Williams RD, Cattini N, et al. Gene expression profiling after radiation-induced DNA damage is strongly predictive of BRCA1 mutation carrier status. *Clin Cancer Res*. 2004;10(3):958–963.
15. Bellacosa A, Godwin AK, Peri S, et al. Altered gene expression in morphologically normal epithelial cells from heterozygous carriers of BRCA1 or BRCA2 mutations. *Cancer Prev Res (Phila)*. 2010;3(1):48–61.
16. Feilotter HE, Michel C, Uy P, Bathurst L, Davey S. BRCA1 haploinsufficiency leads to altered expression of genes involved in cellular proliferation and development. *PLoS One*. 2014;9(6):e100068.
17. Salmon AY, Salmon-Divon M, Zahavi T, et al. Determination of molecular markers for BRCA1 and BRCA2 heterozygosity using gene expression profiling. *Cancer Prev Res (Phila)*. 2013;6(2):82–90.

18. Zahavi T, Yahav G, Shimshon Y, et al. Utilizing fluorescent life time imaging microscopy technology for identify carriers of BRCA2 mutation. *Biochem Biophys Res Commun*. 2016;480(1):36–41.
19. Barwell J, Pangon L, Georgiou A, et al. Lymphocyte radiosensitivity in BRCA1 and BRCA2 mutation carriers and implications for breast cancer susceptibility. *Int J Cancer*. 2007;121(7):1631–1636.
20. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J North Am*. 2011;17(1):10–12.
21. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol*. 2013;14(4):R36.
22. Anders S, Pyl PT, Huber W. HTSeq – a Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2014;31(2):166–169.
23. Smyth GK. Limma: linear models for microarray data. In: Gentleman R, Carey VJ, Huber W, Irizarry RA, Dudoit S, editors. *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*. New York, NY: Springer; 2005:397–420.
24. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol*. 2010;11(3):R25.
25. Law CW, Chen Y, Shi W, Smyth GK. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol*. 2014;15(2):R29.
26. Ben-Ari Fuchs S, Lieder I, Stelzer G, et al. Geneanalytics: an integrative gene set analysis tool for next generation sequencing, RNAseq and microarray data. *Omi A J Integr Biol*. 2016;20(3):139–151.
27. Keen JC, Moore HM. The genotype-tissue expression (GTEx) project: linking clinical data with molecular analysis to advance personalized medicine. *J Pers Med*. 2015;5(1):22–29.
28. Ramsay AJ, Martínez-Trillos A, Jares P, Rodríguez D, Kwarciak A, Quesada V. Next-generation sequencing reveals the secrets of the chronic lymphocytic leukemia genome. *Clin Transl Oncol*. 2013;15(1):3–8.
29. Speir ML, Zweig AS, Rosenbloom KR, et al. The UCSC Genome Browser database: 2016 update. *Nucleic Acids Res*. 2016;44(D1):D717–D725.
30. Repana K, Papazisis K, Foukas P, et al. Expression of Syk in invasive breast cancer: correlation to proliferation and invasiveness. *Anticancer Res*. 26(6C):4949–4954.
31. Barr PM, Wei C, Roger J, et al. Syk inhibition with fostamatinib leads to transitional B lymphocyte depletion. *Clin Immunol*. 2012;142(3):237–242.
32. Fridlich R, Annamalai D, Roy R, Bernheim G, Powell SN. BRCA1 and BRCA2 protect against oxidative DNA damage converted into double-strand breaks during DNA replication. *DNA Repair (Amst)*. 2015;30:11–20.
33. Flück M, Zürcher G, Andres AC, Ziemiecki A. Molecular characterization of the murine syk protein tyrosine kinase cDNA, transcripts and protein. *Biochem Biophys Res Commun*. 1995;213(1):273–281.
34. Vuillaume M-L, Uhrhammer N, Vidal V, et al. Use of gene expression profiles of peripheral blood lymphocytes to distinguish BRCA1 mutation carriers in high risk breast cancer families. *Cancer Inform*. 2009;7:41–56.
35. Zhao S, Fung-Leung W-P, Bittner A, Ngo K, Liu X. Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. *PLoS One*. 2014;9(1):e78644.
36. Prakash R, Zhang Y, Feng W, Jasin M. Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. *Cold Spring Harb Perspect Biol*. 2015;7(4):a016600.
37. Coopman PJ, Do MT, Barth M, et al. The Syk tyrosine kinase suppresses malignant growth of human breast cancer cells. *Nature*. 2000;406(6797):742–747.
38. Coopman PJ, Mueller SC. The Syk tyrosine kinase: a new negative regulator in tumor growth and progression. *Cancer Lett*. 2006;241(2):159–173.
39. Sonnenblick A, de Azambuja E, Azim HA, Piccart M. An update on PARP inhibitors – moving to the adjuvant setting. *Nat Rev Clin Oncol*. 2015;12(1):27–41.
40. Naldi A, Larive RM, Czerwinska U, et al. Reconstruction and signal propagation analysis of the Syk signaling network in breast cancer cells. *PLoS Comput Biol*. 2017;13(3):e1005432.

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