STK3 higher expression association with clinical characteristics in intrinsic subtypes of breast cancer invasive ductal carcinoma patients

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ABSTRACT

Purpose

STK3 has a central role in maintaining cell homeostasis, proliferation, growth, and apoptosis. Previously, we investigated the functional link between STK3/MST2, and estrogen receptor in MCF-7 breast cancer cells. To expand the investigation, this study evaluated STK3's higher expression and associated genes in breast cancer intrinsic subtypes using publicly available data.

Methods

The relationship between clinical pathologic features and STK3 high expression was analyzed using descriptive and multivariate analysis.

Results

Increased STK3 expression in breast cancer was significantly associated with higher pathological cancer stages, and a different expression level was observed in the intrinsic subtypes of breast cancer. Kaplan-Meier analysis showed that breast cancer with high STK3 had a lower survival rate in IDC patients than that with low STK3 expression ($p < 0.05$). The multivariate analysis unveiled a robust correlation between STK3 expression and the survival rate among IDC patients, demonstrating hazard ratios for lower expression. In the TCGA dataset, the hazard ratio was 0.56 (95% CI: 0.34-0.94, $p = 0.029$) for patients deceased with tumor, and 0.62 (95% CI: 0.42-0.92, $p = 0.017$) for all deceased patients. Additionally, in the METABRIC dataset, the hazard ratio was 0.76 (95% CI: 0.64-0.91, $p = 0.003$) for those deceased with tumor. From GSEA outcomes 7 gene sets were selected based on statistical significance (FDR < 0.25 and $p < 0.05$). Weighted Sum model (WSM) derived top 5% genes also have higher expression in basal and lower in luminal A in association with STK3.

Conclusion

By introducing a novel bioinformatics approach that combines GSEA and WSM, the study successfully identified the top 5% of genes associated with higher expression of STK3.

Keywords: Breast cancer, Intrinsic subtype, STK3 higher expression, gene sets (pathways), leading-edge genes.

1. INTRODUCTION

Breast cancer have many potential causes, often including a combination of genetic, hormonal, environmental, and lifestyle factors [6]. The diagnosis and treatment of breast cancer can be achieved by identification prognostic risk factors [32]. Sometimes mutations in certain genes, such as BRCA1 and BRCA2, and abnormality in signaling pathways are linked to an increased risk, which are complex and tightly controlled in normal development and regulations [6]. It has been shown that hippo pathways, which control cell proliferation, growth, and cell differentiation, are dysregulated in breast cancer compared to normal breast [17, 21, 25, 30, 36]. The Hippo pathway is an evolutionarily conserved regulator of tissue growth and cell fate during development, and regeneration and keeps tissues homeostasis. [4, 5, 16]. Mammalian Sterile 20-like kinases (STKs such as STK3 and STK4 which respectively known as MST2 and MST1), large tumor suppressor (LATS) kinases, Salvador homolog 1 (SAV1) scaffolding protein, monopolar spindle-one-binder kinase activator protein 1 (MOB1), and YAP (Yes-associated protein) are the main proteins that make up the canonical Hippo pathway in mammals. Mutation and deregulation for a subset of Hippo pathway genes have been reported in several malignancies, including breast cancer [4]. This relationship aids in the regulation of Lats1/2-Mob1 complexes by MST1/2, which phosphorylates and retains YAP/TAZ in the cytoplasm, inactivating downstream targets [15, 26, 34]. MST1/2 activation has been linked to tumor suppression and apoptosis, according to functional investigations [27, 29]. As a tumor suppressor, MST1 inhibits the spread of tumors and triggers apoptosis in breast cancer [27]. Patients who had diminished MST1 expression in breast cancer had a considerably reduced lifespan compared to individuals with high MST1 expression. There has been hypothesis suggesting MST1 expression is a predictive factor for people with breast cancer [13]. An essential interaction between ER and MST2 in breast cancer is implied by our prior analysis, which showed that the excellent predictive benefit of low MST2 was only detected within ER-positive breast cancer patients as opposed to ER-negative patients. Due to the fact that other cancer types did not exhibit this association.

The purpose of this study is to evaluate the STK3 higher expression patterns in different subtypes and their association with pathological stages of breast cancer patients. Patients were classified into higher and lower expression phenotypes of the STK3 gene using a median-based cut-off expression value.

In this study, the publicly available data The Cancer Genome Atlas (TCGA) and Molecular Taxonomy of Breast

Cancer International Consortium (METABRIC) are analyzed from cBioPortal which can be founded at (https://www.cbioportal.org/) and STK3 expression is associated with the pathological stages and intrinsic subtypes is displayed in descriptive statistical form. The patient's survival analysis is performed using the Kaplan-Meier method. The gene sets from hallmark and breast cancer-related pathways in the context of STK3 high and low phenotypes using Gene Set Enrichment Analysis (GSEA) [24] were analyzed. The statistically significant gene sets (pathways) that were commonly enriched in both datasets were selected, and the leading-edge genes were examined using Weighted Sum Model (WSM) and Nominal Group Technique (NGT) [14] to identify the top 5% of genes associated with STK3. Moreover, a consistent expression pattern was observed across both datasets regarding the distribution of patients among intrinsic subtypes. We concluded that STK3's higher expression has a vital role in overall invasive ductal carcinoma (IDC) patients by indicating that the survivability of breast cancer patients is significantly decreased after five years ($p < 0.05$) compared to its lower expression. Furthermore, based on the clinical-pathological stage association, it has been observed that patients with an advanced stage also exhibit higher expression of STK3. The basal subtype is most strongly associated with higher expression of STK3, while lower expression is correlated with luminal A-type, suggesting a connection between STK3 expression levels and the degree of cancer aggressiveness.

In general, the bioinformatic study of the genomic profile of breast cancer could offer a hint for discovering potential biomarkers and help with treating patients individually based on their gene expression. Based on our study results, it can be inferred that STK3's higher expression and related highly ranked gene expressions have the potential to be candidate biomarkers for intrinsic subtypes, particularly in basal and luminal A breast cancer subtypes that exhibit considerable variation for differential analysis.

2. MATERIALS AND METHODS

2.1. Data collection

Large public databases containing cancer-related data are widely accessible for researchers. The study utilized two such databases, from cBioPortal, which provided access to genomic and clinical data from large studies such as TCGA and METABRIC.

2.1.1. TCGA data collection and preprocessing

The TCGA data underwent various processing steps to ensure consistency and accuracy in subsequent statistical

analyses. Initially, data from 1084 patients (RNA seq count data and clinical data) were retrieved from the TCGA database via cBioPortal. Patients with more than 70% missing clinical data were excluded, and missing clinical features were obtained using the TNM (Tumor, node, and metastasis) staging system data. Unique identifiers - Case-Id for transcriptome data and bcr_patient_barcode for clinical data - were used to map clinical and transcriptomic data. To handle multiple transcriptomes for the same patient, the mean expression of transcriptomic records was used. To ensure an adequate sample size, the study exclusively examined transcriptomes from primary tumor tissues, with the small number of patients (around 5 for metastatic and 113 for solid tissue normal) precluding the analysis of metastatic and solid tissue normal data. The final analysis included transcriptome data from 780 IDC patients. All data processing and analysis were performed using R software.

2.1.2. METABRIC data collection and preprocessing

The METABRIC database, comprising 2509 primary breast tumors and 548 matched normal samples (RNA seq count data and clinical data), was obtained from cBioportal. The samples were uniquely identified using the cancer study identifier brca_metabric. It should be noted that the normal samples only had clinical data and no transcriptomic records. Furthermore, around 57 patients with primary tissue were also missing the transcriptomic data. To ensure the quality of the dataset and retrieve consistent outcomes, we removed a total of 605 samples without transcriptomic data and merged the remaining clinical and transcriptomic data, which were mapped based on the brca_metabric identifier. To conduct our final analysis, we focused on 1500 IDC patients out of the 1904 samples in the dataset.

2.2. STK3 higher expression association with clinical features

2.2.1. Descriptive statistical analysis

Patients in both datasets were categorized into low and high phenotypes based on the STK3 median gene expression value. The patients with STK3 expression less than the median value were considered low phenotype, while those with expression higher than the median value were considered high phenotype. The STK3 expression was visualized in box plots in clinical-pathological stages as well as molecular subtypes of breast cancer using R programming.

2.2.2. Survival analysis

The survival analysis of both datasets was conducted using the Kaplan-Meier method in R, based on the STK3 higher and lower phenotypes. The analysis utilized two parameters: the time from the first diagnosis to the last

follow-up or death, and the status of patients (i.e., alive, or deceased). Furthermore, the Cox regression model was employed to examine the hazard rate of the low and high STK3 categories of patients' data.

2.3. GSEA analysis

To determine whether a set of previously defined genes exhibits statistically significant differences between two biological states, a computational technique known as GSEA is utilized [1]. In this study, GSEA was used to identify the associated upregulated pathways in the STK3 higher expression phenotype. The Hall Mark (h.all.v2022.1.Hs.symbols) and a query-driven gene set using the query *"STK3, breast cancer, tumor microenvironment, nuclear translocation, genes regulations, hippo pathway, hypoxia, cell proliferation"* from Gene card were used for GSEA analysis [11]. Further analysis was performed by selecting pathways that were upregulated in the higher STK3 phenotype and were common to both data sets, with a p-value of less than 0.05 and an FDR of less than 25%. It is worth mentioning that no gene set with a significant p-value and matching threshold FDR value was observed in the STK3 low phenotype.

2.4. Leading edge genes analysis and top 5% genes derivation in association with STK3

After the selection of significant pathways enriched in higher expression of STK3 using a threshold of NES > 1.5, $p < 0.05$, and FDR < 25% were visualized in higher and lower expression context of STK3. To analyze the mean expression of leading edge genes for each subtype, heat map visualizations were used. The violin plots were used to display the expression pattern of significant pathways among breast cancer patients of different subtypes, categorized by SKT3 phenotype as either low or high. The line plots were used to depict the patient percentage for higher mean expression of leading edge genes across all significant pathways in TCGA and METABRIC datasets to observe trends of higher mean expression in the context of STK3's higher phenotype across different subtypes of breast cancer. After all leading edge genes of significant pathways in SKT3 higher phenotypes were subjected to statistical analysis using WSM. The WSM used several features of genes, including the normalized enrichment score (NES) of the gene's pathway, the running enrichment score (RES) of a gene in the gene sets/pathway, the total number of pathways that shared a given gene, and the scaled mean expression of the gene between the TCGA and METABRIC datasets (Table 1). We evaluated two sets of weights (Wt1, Wt2) from our team members closely working on STK3's role in different diseases. The WSM was used to calculate the ranking based on the four criteria (Table 1). In WSM, the weights for each criterion were decided based on

the NGT [14]. NGT is a group process that assists in selecting appropriate solutions to a problem based on the majority group member consensus. The problem was assigning the most moderate weights to criteria based on experts' domain knowledge in the gene ranking process. This model enables the identification of the top 5% of genes that are associated with STK3.

$$Gene\ Ranking_{(WSM)} = \sum_i C_i \times NGT(C_i)$$

$Where\ C_i\ represents\ gene\ ranking\ crteria\ shown\ in\ Table\ 2, i.e.\ C_i\{Gene_{NES}, Gene_{RES}, Gene_{cpw}, Gene_{Exp}\}$

$and\ NGT(C_i)\ is\ a\ weight\ to\ C_i\ \ assign\ through\ NGT\ process.$

Table 1: Gene ranking factors and criteria

Ranking Factors	Selection criteria (C)	Example	NGT	
			Wt1	Wt2
Normalized enrichment score (NES)	$Gene_{NES} = \frac{\sum_{p=1,d}^{n,d\in D} max_p(NES_d)}{\#D}$ $where\ p\ represent\ Gene's\ pathway\ and\ D = \{tcga, meta\}\ \ represent\ datasets - TCGA\ and\ METABRIC$	Consider gene A found in pathways P1 and P2. NES of P1 = (1.2,3.2), P2 = (2.1, 2.0) in TCGA and META respectively. Then $A_{NES} = \frac{3.2 + 2.1}{2} = 2.65$	0.40	0.30
Running Enrichment score (RES)	$Gene_{RES} = max\left(max(RES_p)_D\right)$ $Where\ D = \{tcga, meta\}\ and\ p \subseteq \{1,2,\dots,7\} - Gene's\ pathway$	Consider gene A found in pathways P1 and P2. RES of P1 = (0.3,0.2), P2 = (0.4, 0.1) in TCGA and META respectively. Then $A_{RES} = \max(\max(0.3,0.4), \max(0.2,0.1)) = 0.4$	0.20	0.30
Number of gene sets/pathways(P)	$Gene_{cpw} = \frac{\#(p_i \mid Gene \in p_i\ \&\ i = \{1,2,3,\dots,7\})}{\#(P)}$ $where\ p_i\ is\ individual\ pathway\ number, P\ is\ total\ total\ significant\ pathways$	Consider gene A found in pathways P1 and P2. And we have a total significant number of pathways are 7. Then $A_{cpw} = \frac{2}{7} = 0.28$	0.10	0.10
Gene expression (GE)	$Gene_{Exp} = \frac{\sum median\left(Exp_{gene}(Pat_D)\right)}{\#D}$ $Where\ Pat_D\ represents\ all\ patients\ in\ dataset\ D, and\ D = \{tcga, meta\}$	Consider gene A has median expression 0.7 in TCGA, and 0.5 in METABRIC, Then $A_{Exp} = \frac{0.7 + 0.5}{2} = 0.6$	0.30	0.30

3. RESULTS

3.1. STK3 higher expression association with clinical characteristics

We analyzed STK3's higher expression associated with the pathological stages and the patient's distribution in intrinsic subtypes of breast cancer patients in TCGA and METABRIC datasets.

TCGA data (Figure 1a) shows the patient proportion for STK3 higher expression increase precisely by moving

from pathological lower stage I to higher stages III (I~II: 39% to 49%, II~III: 49% to 61%). In stage IV, the patient distribution is observed lower compared to stage III. So, the results remain inconclusive due to the relatively small proportion of patients (2.18%) in stage IV.

Based on the METABRIC data analysis (Figure 1b), there is a clear increase in the proportion of patients with high-risk STK3 expression as breast cancer progresses from lower stage I to higher stages IV. Specifically, the proportion of patients with high-risk STK3 expression increases from 43% to 51% when moving from stage I to II, from 51% to 56% when moving from stage II to III, and from 56% to 67% when moving from stage III to IV. These findings suggest a positive association between higher STK3 expression and advanced stages of breast cancer.

STK3 is expressed differently amongst intrinsic molecular subtypes of breast cancer in TCGA and METABRIC datasets (Figure 1c and 1d). STK3 higher expression pattern in each subtype for both dataset is given below.

- Patients with Basal subtype showed higher STK3 expression levels in both TCGA and METABRIC datasets, with percentages of 62% and 68%, respectively. Additionally, the patient proportions of the Basal subtype in TCGA and METABRIC were 19.62% and 12.40%, respectively.

- Both TCGA and METABRIC datasets showed a higher level of STK3 expression (58%) in patients with Luminal B subtype. The patient proportions for Luminal B subtype were 19.62% and 12.40% in TCGA and METABRIC, respectively.

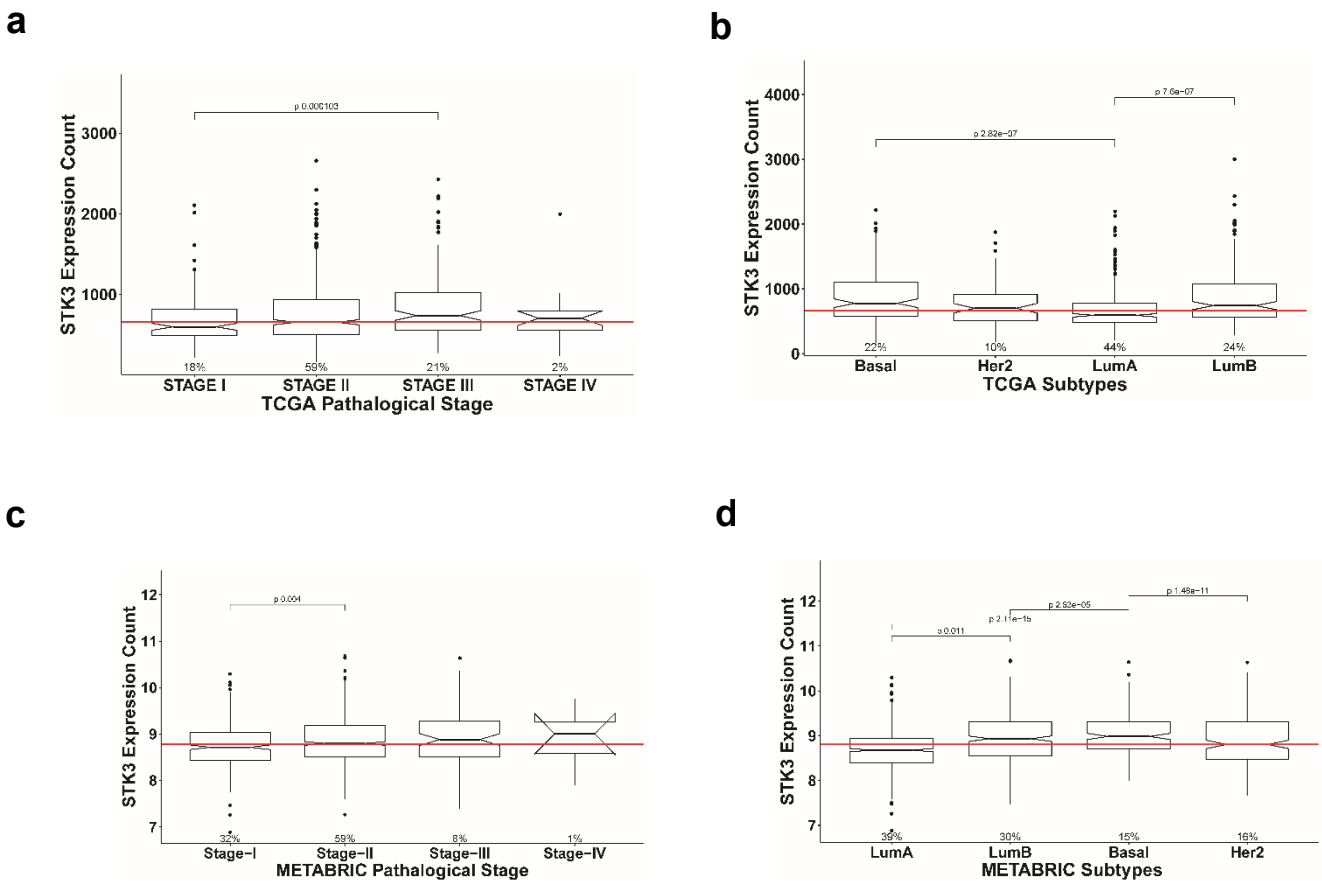
- Both TCGA and METABRIC datasets showed a higher expression of STK3 in 54% of patients (with a ratio of 9.23%) and 52% of patients (with a ratio of 13.00%), respectively in subtype Her2.

- The proportion of patients with Luminal A subtype exhibiting higher STK3 expression was 37% and 40% in TCGA and METABRIC datasets, respectively. Notably, Luminal A patients represented the majority of samples in both datasets, accounting for 49.48% and 50.00% of TCGA and METABRIC samples, respectively.

The results suggest that the expression of STK3 varies among the intrinsic subtypes of breast cancer. Specifically,

STK3's higher expression is observed in the Basal subtype, while it is lower in the Luminal A subtype.

Fig. 1



STK3 expression in pathological stages and subtypes of breast cancer of TCGA dataset (**a, b**) and in pathological stages and subtypes of breast cancer of METABRIC dataset (**c, d**). The distribution of patients in each group is depicted beneath the whiskers of the respective box labels.

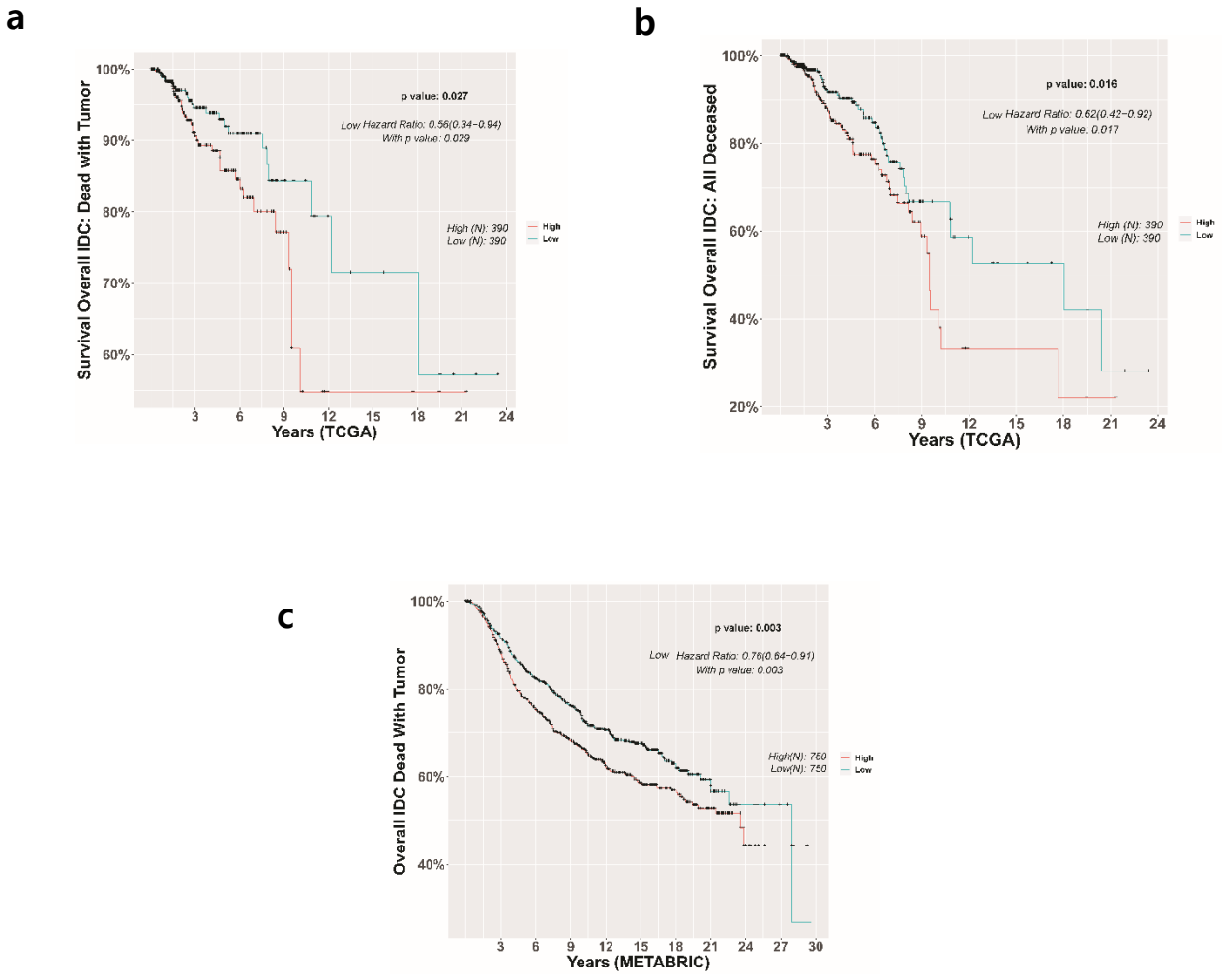
3.2. IDC patients' survival in context of STK3 lower and higher expression

The Kaplan survival analysis for five to ten years shows that breast cancer IDC patients with STK3 higher expression have low survivability compared to STK3 low expression both in TCGA and METABRIC data sets. The survival rates exhibit notable distinctions, with p-values of 0.027 and 0.016 in TCGA for patients classified

as "dead with tumor" and all deceased IDC patients, respectively. In the METABRIC dataset, the survival rate for patients labeled as "dead with tumor" also demonstrates a significant difference with a p-value of 0.003. Figure 2 depicts the details of survival curves in both TCGA (a, b) and METABRIC (c).

The survival rate of IDC patients significantly correlates with STK3 expression, as indicated by multivariate analysis. In the TCGA dataset, lower expression shows a hazard ratio of 0.56 (95% CI: 0.34-0.94, p = 0.029) for patients who died with tumors and 0.62 (95% CI: 0.42-0.92, p = 0.017) for all deceased patients. In the METABRIC dataset, the hazard ratio for those deceased with tumors is 0.76 (95% CI: 0.64-0.91, p = 0.003).

Fig.2



Survival plot STK3 low and high expression TCGA (**a, b**) METABRIC (**c**).

3.3. Candidate Gene sets regulations in context of STK3 low and high expression

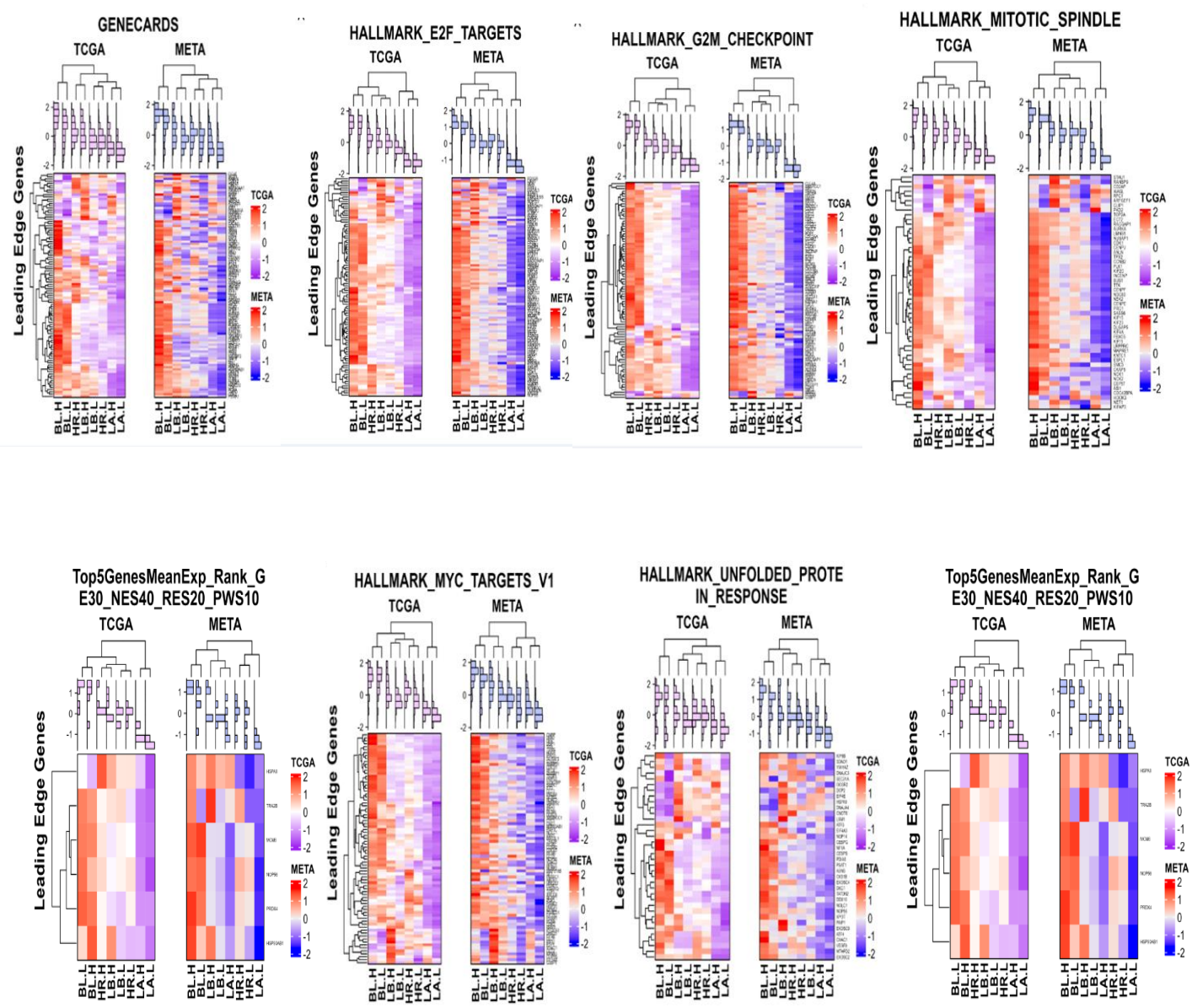
We get the most enriched pathways in both datasets with a p-value less than 0.05 and an FDR value <25% using GSE analysis. A total of 7 pathways are commonly enriched in both datasets. The 7 Pathways (Supplementary Figure S1) shows only pathways from which the top 5% of genes are associated with STK3 expression.

To identify signaling pathways that are differentially activated in breast cancer in both TCGA and METABRIC, GSEA was conducted between low and high STK3 expression data sets. GSEA reveals significant differences (FDR 0.25 %, NOM p-value % 0.05) in the enrichment of MSigDB Collection (h.all. v2022. Symbols [12]). We selected the most significantly enriched signaling pathways based on their NES (Supplementary Figure S1 and Supplementary Table S1). In supplementary Figure S1 shows that GM2_check point, E2F targets, mitotic spindle, MTORC1 signaling, MYC targets V1, and unfolded_protein_response is differentially enriched in STK3 high expression phenotype. Hallmarks can effectively associate with their corresponding protein activation phenotypes thus confirming their biological relevance.

3.4. Leading edge genes Visualization in STK3 context

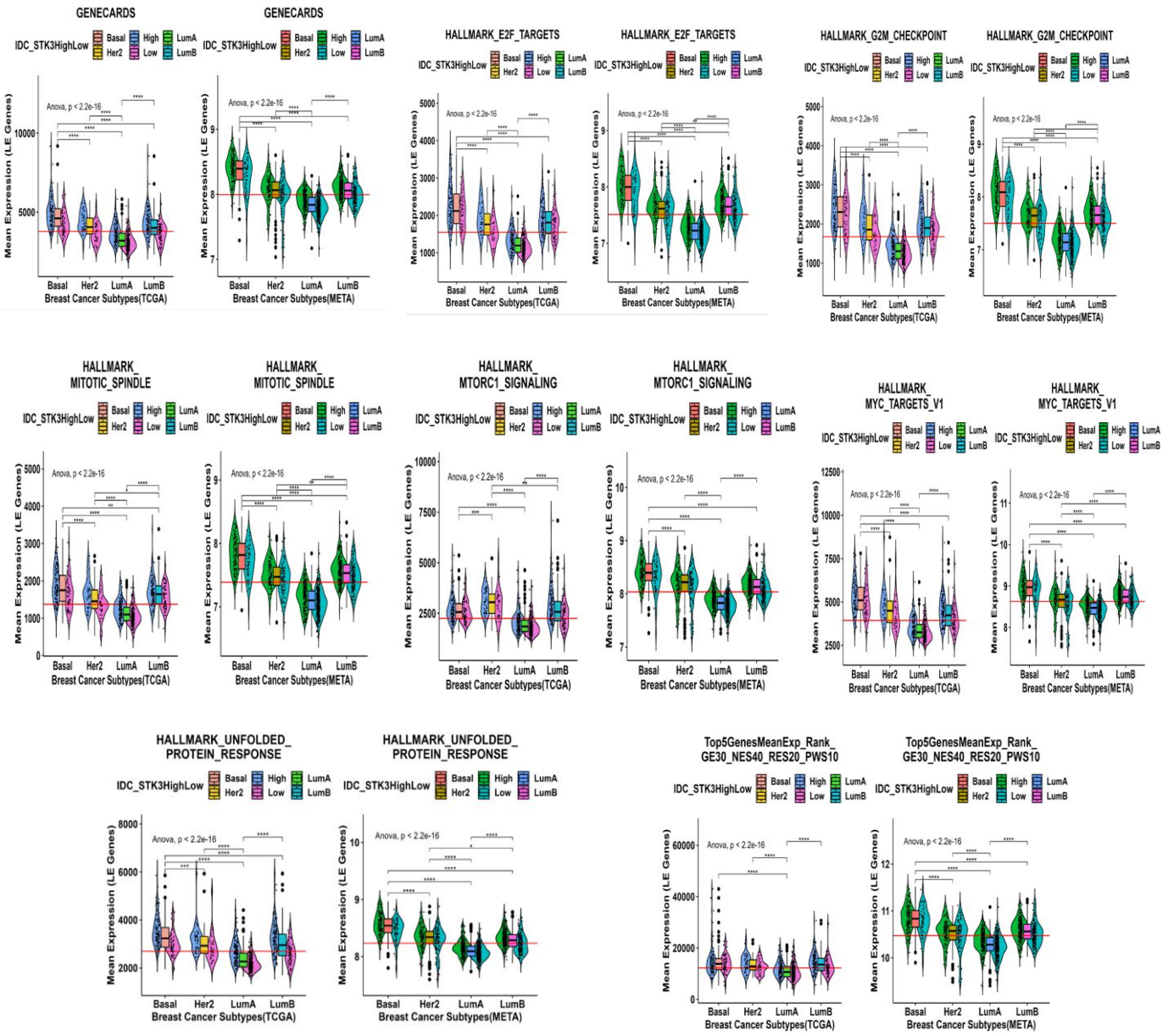
The seven significant gene sets and one selected top 5% gene in the context of STK3 low and higher phenotype and in different subtypes of breast cancer were visualized using heat maps and violin plots to display the leading edge genes. The results showed that for all significant gene sets, patients had a higher percentage of higher expression for the leading edge genes in the basal subtype and a lower percentage in Luminal A. This trend was observed in both data sets, TCGA and METABRIC (Figure 3). [Higher resolution is shown in supplementary figure 3(a)]

Fig.3(a)



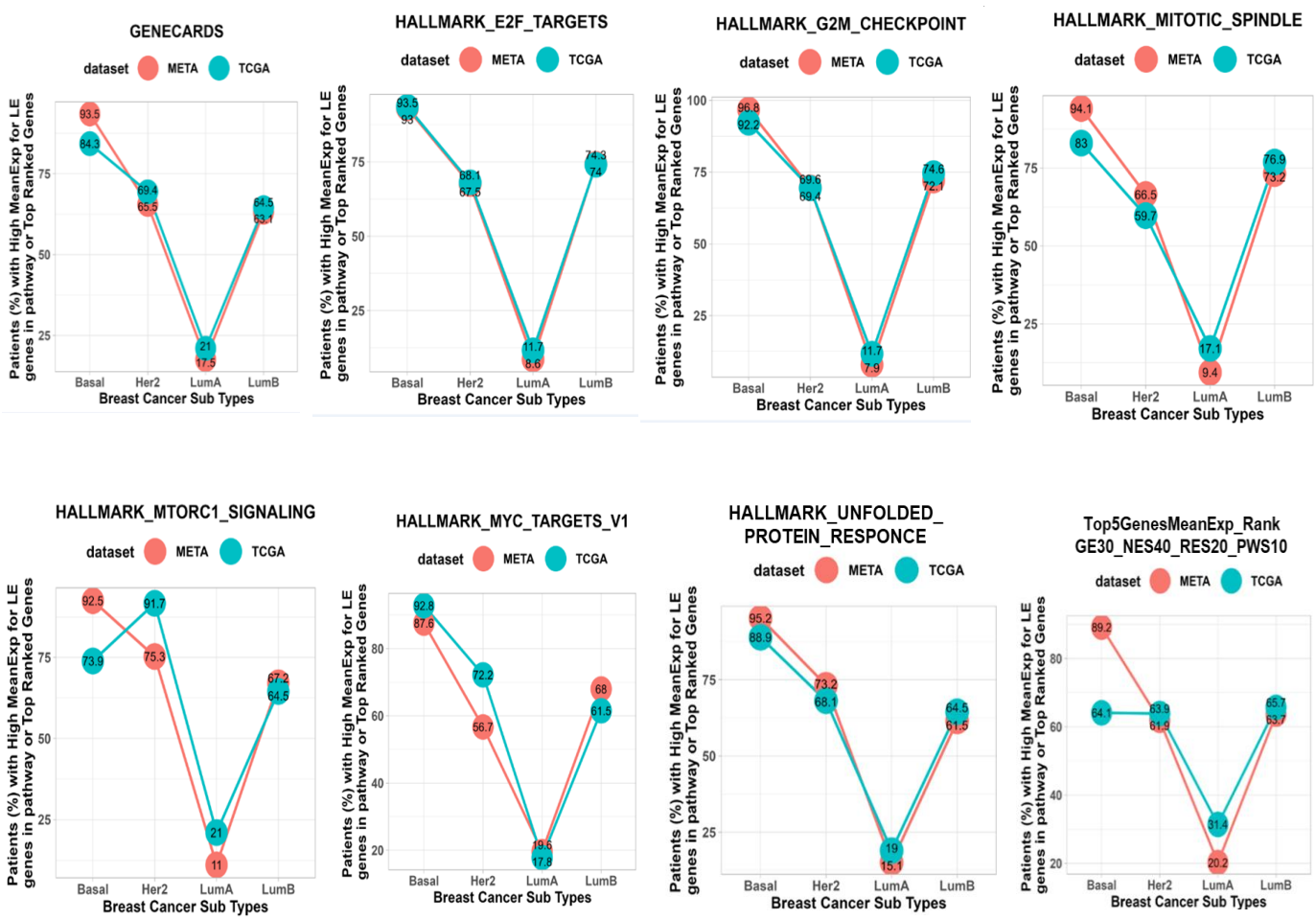
Heatmap Visualization of leading-edge genes (LEG) for statistically significant gene sets and the top 5% genes, in the context of STK3 in breast cancer subtypes [(BL: Basal, HR: HER2, LA: Luminal A, LB: Luminal B) ;(L: lowSTK3Expression, H: HighSTK3Expression)] of TCGA and METABRIC data sets.

Fig.3(b)



LEG mean expression distribution across subtypes in the context of STK3 using data from TCGA and METABRIC

Fig.3(c)



Patterns of Patients percentage of LEG with high mean expression of statistically significant gene sets, in breast cancer subtypes Of TCGA and METABRIC datasets.

3.5. Top 5% genes derivation in association with STK3

The WSM model derived the top 5% genes associated with STK3 relied on GSEA-derived features such as NES, RES, pathways (gene sets) number, and gene expression from patients TCGA and METABRIC data sets. The genes that were identified using this approach could provide valuable insights into the underlying mechanisms of breast cancer and may ultimately aid in the development of more effective treatments for this disease. These top 5 % genes are HSPA8, HSP90AB1, NOP56, MCM6, TRA2B, and PRDX4, and have literature evidence to

have a role in breast cancer proliferation [3, 7, 8, 20, 22]. Table 3 highlights the detailed features of each of the top 5% genes with overall score and ranks. Although, Wt1 score criteria were used, Table 2 also provided the gene score and overall score and ranks using Wt2 criteria.

Table 2: Top 5% ranked genes list.

Gene	Average Median GExp Scaled	Max Pathways NES	Mean RES	PW Relative Score	NGT Score	
					Wt1 | Rank	Wt2 | Rank
HSPA8	0.91	1.00	0.70	0.4	0.861 | 1	0.865 | 1
HSP90AB1	1.00	0.94	0.35	0.4	0.783 | 2	0.743 | 8
MCM6	0.47	1.00	0.71	0.6	0.781 | 3	0.778 | 3
TRA2B	0.49	1.00	0.68	0.6	0.781 | 4	0.775 | 4
PRDX4	0.59	0.99	0.66	0.4	0.780 | 5	0.772 | 5
NOP56	0.51	0.99	0.72	0.6	0.776 | 6	0.780 | 2

4. DISCUSSION

The Hippo pathway component has a substantial role in regulating the cell cycle, growth, proliferation, and maintaining tissue homeostasis. Furthermore, it inhibits the development and occurrences of malignancy tightly controlled under normal conditions depending on the types of signaling. In a study of human sarcoma tumorigenesis, the epigenetic alteration effect was observed for STK3(MST2) in signaling pathway of Sav-RASSF1-Hpo.[23]. Furthermore, STK3's lower expression is correlated with poor prognosis in ovarian cancer, and higher expression inhibits the cell proliferation, and migration of ovarian cancer cells and promotes apoptosis [28].

In a study of gastric carcinogenesis, STK3 was discovered to be an independent prognostic biomarker that mediates cell cycle progression by activating Ras-MAPK pathways [1].

Our previous work has proved that the expression of two components STK3 (MST2) and SAV of the hippo pathway was associated with ERα phosphorylation and transactivation and represses ERα gene expression. Silencing of STK3 can inhibit breast cancer in vitro experiments using MCF-7 cells and showed that its higher expression leads to ERα activation in the absence of ligand [18].

We aimed to analyze STK3 as a potential prognostic molecular marker of poor survival. Bioinformatic analysis in this study showed that STK3 has higher expression levels in basal types and lower expression in luminal A

type of breast cancer patients. Furthermore, its higher expression is closely related to the poor prognosis of IDC breast cancer patients.

We observed that the STK3 higher expression is associated with higher stages in both datasets, but for only TCGA data, the stage IV patients had comparatively low STK3 expression. One possible reason could be a smaller number of patients compared to other stages, or different biological and molecular mechanisms are involved.

By using the statistical model WSM and NGT based ranking on GSEA-derived features such as NES, enrichment score, and pathways number the top 5 % genes were derived in STK3 higher phenotype-based GSEA leading edge genes. The genes that were identified using this approach could provide valuable insights into the underlying mechanisms of breast cancer and may ultimately aid in the development of more effective treatments for this disease. For example, HSPA8, HSP90AB1, NOP56, MCM6, TRA2B, and PRDX4, were identified as genes that were analyzed in STK3 higher expression phenotype study. These genes have already been observed through some preliminary studies which are evidently having a role in breast cancer proliferation [3, 7-9, 20].

One study indicates the potential molecular mechanism that promotes the evolution of TNBC (triple negative breast cancer) related with the poor clinical outcome of TNBC is associated with high expression of HSPA8 [31]. High-level expression of HSP90AB1, one of cytoplasmic HSP90 isoforms was correlated with poor prognosis in different subtypes of breast cancer and was driven by chromosome coding region amplifications and were independent factors that led to death from breast cancer among patients with triple- negative (TNBC) and HER2-/ER+ subtypes [2]. MCM6 is known as a specific biomarker of cancer in many cancer types including breast cancer. its expression level, and biological function in various types of cancer is complicated and have remain uncleared up to date [33]. TRA2B is in association with several other genes and its product involve in breast cancer metastasis and was identified as cancer hall mark [19]. PRDX4 antioxidant protein has been shown to causally facilitate tumor initiation and propagation, therapeutic resistance, and subsequent recurrence of many types of tumors. The mechanisms of how PRDX4 works in different cancers requires more in depth research [10]. NOP56 is located at the key crossroads of many signaling pathways and plays an important role related to the occurrence and development of various tumors. A although the role and mechanism of NOP56 are still unclear. However, it is one of many methylated genes, and examining the methylation status of genes can help identify

tumor-specific markers and therapeutic targets for cancer patients [35].

This finding suggests that these genes may play a role in the development of breast cancer in patients with high levels of STK3 expression. This study is novel to exploit STK3 expression in different intrinsic subtypes of breast cancer at the more granular level using two data sets. Consideration of underlying molecular mechanisms in association with hippo pathways genes especially STK3 could lead to develop the targeted therapy for a more aggressive type like basal or triple-negative cancer type of breast cancer and improve the patient's life quality.

Several studies have implied the NGT to involve stakeholders and gather their views and opinions to develop consensus-based healthcare decisions [14]. Some common examples include establishing end-of-life care preferences, prioritizing treatment decisions, highlighting chronic disease issues, and developing research-based guidelines. This work employed NGT techniques to rank genes and reach a consensus on weighting gene attributes retrieved from GSEA. Bioinformaticians, and medical data scientists determined WSM model weights for Wt1 and Wt2, consequently finalized through NGT process. Wt1 was selected using consensus based NGT integration.

5. CONCLUSION

STK3 has been studied in breast cancer as a potential prognostic molecular marker of poor survival. This study is novel to exploit STK3 expression in different intrinsic subtypes of breast cancer at the more granular level using two data sets. The weighted sum statistical model based on GSEA-derived leading edge genes in STK3 higher phenotypes are the genes having evidence in breast cancer proliferation. Consideration of underlying molecular mechanisms in association with hippo pathways genes especially STK3 could lead to develop the targeted therapy for a more aggressive type like basal or triple-negative cancer type of breast cancer and improve the patient's life quality.

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Statements and Declarations

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

All authors contributed to the study conception and design. Data curation, Methodology, Formal analysis, and Writing – original draft was performed by [Rukhsana]. Data curation, Writing – review & editing were performed by [Afia Tasnim Supty]. Conceptualization, Methodology, Writing – review & editing were performed by [Maqbool Hussain] and Conceptualization, mentoring, Writing – review & editing were performed by [YoungJoo Lee]. All authors read and approved the final manuscript.

Data Availability

The data used in this study is openly accessible and can be found at https://www.cbioportal.org/datasets. Specifically, the following datasets were employed:

1- Breast Invasive Carcinoma (TCGA, PanCancer Atlas 2018)

2- Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016)

Ethics approval

Not applicable

Consent to participate.

Not applicable