

Original Article

Running Title: Genetic Alterations in *PIK3CA* Interacting Network

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Deciphering the Genetic Alterations in *PIK3CA* Gene Interacting Network and Their Putative Association with Breast Cancer: A Computational Approach

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Abstract

Background: Cancer is a polygenic complex disorder involving a network of genes. The phosphatidylinositol 3-kinase (*PIK3CA*) has been reported as an oncogene that plays a role in many cancer types. The present study aims to demonstrate the association between the genetic alterations observed in the *PIK3CA* gene network and its role in establishing breast cancer.

Method: In the present observational study, we used multiple tools (STRING, cBioportal, PANTHER, and UALCAN) to demonstrate the genetic alterations in the Breast Cancer Dataset (TCGA, Firehose Legacy). The *PIK3CA* gene interaction network was deduced, followed by the identification of genetic alterations, gene ontology, gene expression and survival analysis.

Results: The *PIK3CA* gene was found to harbor 36% of genetic alterations in the form of gene amplification and mutations. The gene expression profile indicated the significant downregulation of *PIK3CA* gene transcripts. Interestingly, the Kaplan Meier survival analysis demonstrated that low/medium expression of *PIK3CA* presented with a good prognosis when compared with the high expression group. These results support the fact that *PIK3CA* is oncogenic.

Conclusion: The *PIK3CA* gene has been considered as one of the potential druggable targets for breast cancer. The genetic alterations reported in the gene might influence its function. Therefore, further experimental validation is required to provide more insight into the functional association of mutations. Also, the effect of tumor suppressors and epigenetic factors targeting *PIK3CA* has to be assessed to gain more insight into the increased expression of *PIK3CA* in breast cancer patients.

Keywords: Breast neoplasms, Candidate gene, Mutation, Gene ontology

Introduction

Cancer, as a complex polygenic disorder with a heterogenous phenotype, arises primarily due to aberrations confined to DNA, which in

turn is transcribed and translated into proteins. Gross chromosomal abnormalities, such as deletions and/or duplications, markedly affect the expression profile of the gene, which in

turn contributes to the expression of the proteins.¹ According to the latest GLOBOCAN 2022 report, breast cancer in women has been identified as the most prevalent type of cancer worldwide, surpassing even lung cancer.² The 2020 data indicates an incidence of 2.3 million cases and 0.68 million deaths, which is predicted to increase to 3 million new cases and 1 million deaths by the year 2040.³ Despite the availability of several treatment modalities including chemo, radio, immune and targeted therapies, the overall 10-year survival rate remains more than 90% for developed and 66-79% for developing nations.⁴ The survival of patients is largely affected by the resistance⁵ and recurrence⁶ mediated by several molecular pathways involving important gene networks. In line with these facts, the interacting genes of oncogenic pathways have to be studied to gain more insight into the process of drug resistance and recurrence. The PIK3 pathway is a vital network including a group of lipid kinases that serve as upstream activators of signaling pathways. The alterations in the phosphatidylinositol 3-kinase (*PIK3CA*) gene are associated with solid malignancies presenting with poor prognosis.⁷ The *PIK3CA* is one such gene that encodes the enzyme phosphatidylinositol-3-kinase, which has several cellular functions such as promoting cell transformation, initiation and progression of the tumor, and conferring refractoriness to the process of apoptosis. They are responsive enzymes triggered by growth factors and hormones. The dysregulation of *PIK3CA* activity has been documented in several cancer types including breast cancer.⁸ Gene mutations and other gross chromosomal abnormalities confer oncogenic activity to solid tumors. Mutation analysis is a process that enables researchers to identify mutations, leading to a deeper comprehension of the underlying genetic factors contributing to cancer. These mutations can be classified into two types: driver mutations, and passenger mutations. Driver mutations directly contribute to the development of cancer while passenger mutations are present but do not play a significant role in driving cancerous growth.⁹ Targeted therapies are designed to selectively attack cancer cells with specific genetic

mutations. This approach is intended to reduce damage to healthy cells while increasing the effectiveness of the treatment with minimal side-effects.¹⁰

In line with the facts discussed, the present study aims to demonstrate the effect of genetic alterations in the *PIK3CA* gene and the associated clinical outcome. The novelty of the study lies within the identification of genetic alterations in the primary network of *PIK3CA*, as it would provide more insights into *PIK3CA*-network mediated tumorigenesis. The assessment of mutation frequencies in the circulating tumor DNA is one of the emerging techniques to monitor the treatment response and management of disease in cancer patients. It is one of the widely accepted methods of liquid biopsy employed for screening high-risk groups of individuals to detect cancer at an early stage.¹¹ As cancer is a heterogeneous disorder, the identification of mutations in multiple genes of vital pathways associated with the disease would aid in developing diagnostic panels intended for the early diagnosis of breast cancer. Mutation panels for colorectal cancer,¹² gastric¹³ and lung cancer¹⁴ are available for *in vitro* diagnosis. The present study aims to demonstrate the genetic alterations in the *PIK3CA* interacting network and their role in establishing breast cancer.

Materials and Method

Sample dataset

The dataset used in this observational study was Breast Invasive Carcinoma (TCGA, Firehose Legacy), which included clinical and molecular data of 1085 female and 12 male patients. The data were found unavailable for 4 individuals. The type of neoplasm, race category, age and mutation count are given in table 1.^{15, 16} Out of the 1097 samples collected, data relating to copy number variation and mutation profile were only available for 963 patients, which account for 88% of breast cancer cases.

STRING analysis

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 11.5 is an extensively used online database and web resource for investigating protein-protein interactions (PPIs) (Figure 1). The database provides comprehensive information on PPIs,

including predicted and experimentally verified interactions, functional annotations, and contextual information such as co-expression, co-occurrence, and pathway information. All of the association evidence in the STRING database is categorized into gene neighborhoods, gene fusions, gene co-occurrence, co-expression, experiments, databases and text mining (<https://string-db.org/>, accessed online on 24.04.2023).¹⁷

Gene ontology

The PANTHER database (v16.0; Protein ANalysis THrough Evolutionary Relationships) was used to perform the gene ontology analysis. The gene ontology analysis elaborates on the biological process, molecular functions and sub-cellular localization of gene products. User-defined query lists of genes from each of the datasets were fed as a batch to identify the functional classification of the genes. Pathway-based classification was conducted to identify potential pathways associated with genes.^{18, 19}

OncoPrint data analysis

The cBioportal database (<http://cbioportal.org>) is a platform consisting of clinical and molecular data from multiple cancer types. The OncoPrint algorithm acquires the genomic data for a cohort selected as the input against queried gene/genes. The genomic data are then organized into a matrix where rows represent individual patient samples. The genetic alterations, in the form of mutations and gross abnormalities, can be analyzed through this portal.^{15, 16}

Gene expression and survival analysis

The UALCAN (<http://ualcan.path.uab.edu>) is a user-friendly web portal employed to perform an extensive analysis of a queried gene using TCGA gene expression data. The survival analysis was performed to generate the Kaplan-Meier survival plot. The data were organized into two groups for further analysis: (a) low/medium expression, and (b) high expression. The survival plots were generated using “survival” and “survminer” packages which were further compared by log-rank test. The Survival package is an R software package used for survival analysis. Also, Survminer provides additional features for visualizing and interpreting the results of survival analysis.²⁰

Results

STRING analysis

The *PIK3CA* interacting network consisted of *ERBB3*, *PIK3R1*, *PIK3R2*, *PIK3R3*, *PTEN*, *IRS1*, *KRAS*, *EGFR*, *HRAS* and *AKT1* (Table 2). This complex network has 11 nodes, 55 edges and a PPI enrichment value of 8.08×10^{-7} . The nodes indicate proteins interacting in a specific network. The edges represent protein-protein interactions such as physical, enzymatic or genetic. The PPI value indicates the strength or confidence of protein-protein interaction. These values are used to estimate the likelihood of an interaction between two proteins. The greater the PPI values, the stronger the evidence of an interaction.

Gene ontology

Gene ontology analysis revealed multiple pathways in which the genes are involved. The PIK3 kinase pathway included nine genes: *PIK3CA*, *PIK3R1*, *PIK3R2*, *PIK3R3*, *HRAS*, *AKT1*, *PTEN*, *KRAS* and *IRS1*. Interestingly, genes with the highest frequency of gene dysregulation were included in this cluster. Apart from this pathway, other pathways lead to carcinogenesis such as Ras, PDGF, TGF beta, VEGF, p53 feedback loops and EGF signaling pathways (Figure 2).

OncoPrint data analysis

The highest frequency of gene alteration was demonstrated by *PIK3CA* (36%), followed by *PTEN* (9%) and *KRAS* (3%). All other genes were found to exhibit gene alterations in the range of 1.2 - 2.8% (Figure 3). The somatic mutation frequency was found to be 30.67%. The driver mutations of type missense, nonsense, inframe, splice site, amplification and deep deletion were identified. Gene amplification and deep deletion were found in about 3.12% and 0.1% of cases.

The H1047R/L/Y missense mutation was found to occur at a greater frequency in comparison with other mutations. The pathogenic missense driver mutations occurring in various domains of the *PIK3CA* gene are given in table 3. Truncating mutations and deletions were common with the *PTEN* gene (Figure 4). Multiple alterations were identified in 20.8% of cases.

Gene expression and survival analysis

The gene expression profile of the *PIK3CA* gene in breast invasive carcinoma

demonstrated significant downregulation in the primary tumor group (1.62×10^{-12}) (Figure 5).

Surprisingly, this under-expression of *PIK3CA* did not correlate significantly with the survival status of breast cancer patients. The high-expression group presented with a poor prognosis when compared with the low/medium-expression group. This observation necessitates the investigation of those components which retain the high expression profile in some patients (25%) while reducing the expression in the majority of patients (75%).

Discussion

In the present study, the OncoPrint data analysis showed mutations and gross chromosomal abnormalities such as deletions and amplifications. The present study demonstrated 30.67% of genetic alteration, followed by 3% gene amplification and 0.1% deep deletion. Apart from these mutations inframe, splice-site, and truncating type of mutations were also observed. The inframe mutations can drastically affect protein domains, and binding sites or interfere with the post-translational modifications, whereas splice-site mutations result in altered splicing which results in the production of different isoforms of proteins. The truncating mutations, on the other hand, produce abnormal proteins that are not completely synthesized; therefore, they lack structural as well as functional properties. The mutations are broadly classified into two types: (a) putative driver mutations, and (b) mutations of unknown significance. The former is considered to be the genetic alteration predicted to play a significant role in the development of cancer (Figure 2). They are known to affect the functions of the protein encoded. The analysis of mutation frequencies and prediction of functions based on pathways and networks could enhance the understanding of these putative drivers. The latter refers to those nucleotide substitutions or variants that must be functionally validated to ascertain their functional and clinical relevance. The genetic alterations were predominantly missense mutations, with 18 potentially pathogenic driver mutations. Interestingly, the H1047R

mutation was observed in about 126 breast cancer patients. Although a variety of genetic alterations were found in the *PIK3CA* gene, it did not align with the gene expression profile. The primary tumor group showed a notably low level of expression. The decreased expression of the *PIK3CA* gene, a proto-oncogene was the key observation in the breast cancer dataset selected for the present study. A potential explanation behind the downregulation of the *PIK3CA* gene could be the epigenetic processes such as methylation, modification of histone proteins and target degradation by microRNAs. Furthermore, the study throws light on the genes encoding proteins of the *PIK3CA* network: *ERBB3*, *PIK3R1*, *PIK3R2*, *PIK3R3*, *PTEN*, *IRS1*, *KRAS*, *EGFR*, *HRAS* and *AKT1*. Gene ontology analysis revealed about 27 inter-connect pathways including crucial networks involved in the process of carcinogenesis (Figure 1).

The *PIK3CA* gene mutations have been extensively studied in several types of cancer. The mutation frequency observed was found to be in close agreement with that reported by Martínez-Sáez et al., where they used plasma circulating tumor DNA for mutation analysis. They reported a single mutation in 17 patients (37%) and *PIK3CA* double mutations in one patient (6%) out of 48 cases diagnosed with breast cancer.²¹ Identification of hotspot mutations is crucial for developing therapeutic molecules in order to specifically target mutant proteins that are potentially oncogenic. Liu et al. conducted one such study where they employed computational approaches to identify therapeutic leads for H1074R mutated *PIK3CA* protein. They identified two compounds of ZINC000004098448 and ZINC000014715656 against *PIK3CA* H1074R for triple-negative breast cancer patients.²² *PIK3CA* mutations are common in breast cancer, but not all tumors with these mutations respond to PI3K inhibitors. Correia et al. analyzed tumors from the METABRIC and TCGA projects which demonstrated frequent allelic expression imbalances, with preferential expression of the mutant allele associated with a poorer prognosis, especially in ER-negative, PR-negative, and HER2-positive tumors. This study proposes a novel

model for gene regulation in breast cancer and highlights the clinical relevance of *PIK3CA* allelic expression for aiding in prognosis and identifying patients who are less likely to benefit from PI3K inhibitors.²³

Palimaru et al. investigated the expression of phosphatidylinositol-3-kinase (PI3K) pathway regulators, *PIK3CA* and *PTEN*, in breast carcinoma and normal breast tissue, and their potential association with lymph node metastases in primary breast cancer. Paired samples from 175 patients revealed that the *PIK3CA* and *PTEN* mRNA expression were significantly elevated in breast carcinoma tissue. *PIK3CA* mutations were present in 39% of patients but were not linked to its expression. Interestingly, neither *PIK3CA* nor *PTEN* expression nor *PIK3CA* mutations showed associations with lymph node metastases. The findings suggest that while *PTEN* and *PIK3CA* are dysregulated in breast cancer, their expression and mutations may not directly correlate with lymph node involvement.²⁴ This observation correlated well with the observations made in the present study. Another study, conducted by Alowiri et al., demonstrated the role of the PI3K pathway in breast cancer by analyzing the expressions of its key regulators, *PIK3CA* (activator), and *PTEN* (inhibitor), in breast carcinoma and adjacent normal tissue. The study found that the mRNA levels of both *PIK3CA* and *PTEN* were significantly higher in breast cancer tissue compared with normal tissue as assessed by the Quantitative Real-Time PCR.²⁵ The computational approach has largely contributed to the identification of hot spot mutations in candidate genes²⁶ or complex pathways^{27,28} associated with a specific cancer type. These studies provided clues about convergent pathways²⁹ and gene networks associated with a particular cancer type.³⁰ Taken together, the selection of candidate genes for cancer diagnosis or therapy should be based on the mutational status, transcript load, functions of the protein and epigenetic components, as one factor influences the activity of the other.

Like other computational analyses, the approach provides only preliminary data that should be further validated using experimental approaches to derive a causal relationship.

Given the varied phenotypes of cancer cells based on molecular data, it is important to consider factors such as population, habits, exposures, and lifestyle when interpreting the findings of the present study. Therefore, a more comprehensive approach would be beneficial in developing mutation panels and targeted therapies aimed at addressing breast cancer.

Conclusion

Genetic alterations are the major contributor to genome instability occurring in cancer cells. Therefore, it is of utmost importance to analyze the effect of genetic alterations to demonstrate the role of defective genes in cancer. Despite the significant differential expression of the *PIK3CA* gene in breast cancer patients, high expression presented with a poor prognosis. This instigates the need for exploring epigenetic mechanisms regulating the gene expression process. Targeting those epigenetic marks can open novel avenues for designing and developing therapeutic strategies for breast cancer.

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Conflict of Interest

None declared.

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Table 1. Demographic data of breast cancer patients extracted from Breast Invasive Carcinoma (The Cancer Gene Atlas, Firehose Legacy)

Gender	Female:1085 Male: 12 Data not available: 4
Mutation count	0-4271
Age of diagnosis	26-90
Cancer type	Breast cancer: 1093 Breast cancer, NOS: 7 Skin cancer, Non-melanoma: 1
Neoplasm	Infiltrating ductal carcinoma: 784 Infiltrating lobular carcinoma: 203 Mixed histology: 30 Mucinous carcinoma: 17 Metaplastic carcinoma: 9 Medullary carcinoma: 6 Infiltrating carcinoma NOS: 1 Others: 46 Not available: 5
Race category	White: 757 African: 183 Asian: 61 American Indian: 1 Data not available: 99

Table 2. Genetic alterations observed in the *PIK3CA* interacting genes in Breast Invasive Carcinoma Dataset [The Cancer Gene Atlas, Firehose legacy]

Gene	Protein	Alteration (%)
<i>PIK3CA</i>	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	36
<i>ERBB3</i>	Receptor tyrosine-protein kinase erbB-3	2
<i>PIK3R1</i>	Phosphoinositide-3-kinase regulatory subunit alpha	2.8
<i>PIK3R2</i>	Phosphoinositide-3-kinase regulatory subunit beta	2.1
<i>PIK3R5</i>	Phosphoinositide-3-kinase regulatory subunit gamma	1.5
<i>PTEN</i>	Phosphatase and tensin homolog	9
<i>IRS1</i>	Insulin receptor substrate 1	1.2
<i>KRAS</i>	GTPase KRas	3
<i>EGFR</i>	Epidermal growth factor receptor	2.7
<i>HRAS</i>	GTPase HRas	1.6
<i>AKT1</i>	RAC-alpha serine/threonine-protein kinase	2.1

PIK3CA: Phosphatidylinositol 3-kinase

Table 3. The list of pathogenic driver mutations identified in the *PIK3CA* gene

Mutation	Domain	Frequency
Q546R	Phosphoinositide 3-kinase family, accessory domain (PIK domain) (520 - 703)	6
E545A	Phosphoinositide 3-kinase family, accessory domain (PIK domain) (520 - 703)	2
H1047R	Phosphatidylinositol 3- and 4-kinase domain	126
H1047L	Phosphatidylinositol 3- and 4-kinase domain	12
E542K	Phosphoinositide 3-kinase family, accessory domain (PIK domain) (520 - 703)	40
H1047Y	Phosphatidylinositol 3- and 4-kinase domain	1
R88Q	PI3-kinase family, p85-binding domain (32 - 108)	2
E365K	Phosphoinositide 3-kinase C2 (351 - 483)	1
E453K	Phosphoinositide 3-kinase C2 (351 - 483)	7
C420R	Phosphoinositide 3-kinase C2 (351 - 483)	3
N345K	Phosphoinositide 3-kinase C2 (351 - 483)	15
G118D	PI3-kinase family, p85-binding domain (32 - 108)	4
N345T	Phosphoinositide 3-kinase C2 (351 - 483)	1
V344M	Phosphoinositide 3-kinase C2 (351 - 483)	1
K111E	PI3-kinase family, p85-binding domain (32 - 108)	1
E726K	Phosphoinositide 3-kinase family, accessory domain (PIK domain) (520 - 703)	9
E81K	PI3-kinase family, p85-binding domain (32 - 108)	2
Y1021C	Phosphatidylinositol 3- and 4-kinase (798 - 1014)	1

PIK3CA: Phosphatidylinositol 3-kinase

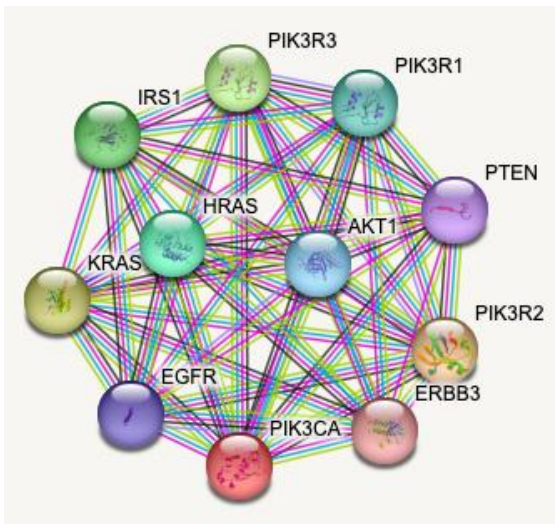


Figure 1. This figure shows the protein-protein network interactions of *PIK3CA* (Phosphatidylinositol 3-kinase) protein as elucidated by the STRING tool.

- Angiogenesis (P00005)
- Apoptosis signaling pathway (P00006)
- Axon guidance mediated by netrin (P00009)
- B cell activation (P00010)
- CCKR signaling map (P06959)
- Cadherin signaling pathway (P00012)
- EGF receptor signaling pathway (P00018)
- Endothelin signaling pathway (P00019)
- FAS signaling pathway (P00020)
- FGF signaling pathway (P00021)
- Gonadotropin-releasing hormone receptor pathway (P06664)
- Huntington disease (P00029)
- Hypoxia response via HIF activation (P00030)
- Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
- Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade (P00032)
- Insulin/IGF pathway-protein kinase B signaling cascade (P00033)
- Integrin signalling pathway (P00034)
- Interleukin signaling pathway (P00036)
- PDGF signaling pathway (P00047)
- PI3 kinase pathway (P00048)
- Ras Pathway (P04393)
- T cell activation (P00053)
- TGF-beta signaling pathway (P00052)
- VEGF signaling pathway (P00056)
- p53 pathway by glucose deprivation (P04397)
- p53 pathway feedback loops 2 (P04398)
- p53 pathway (P00059)

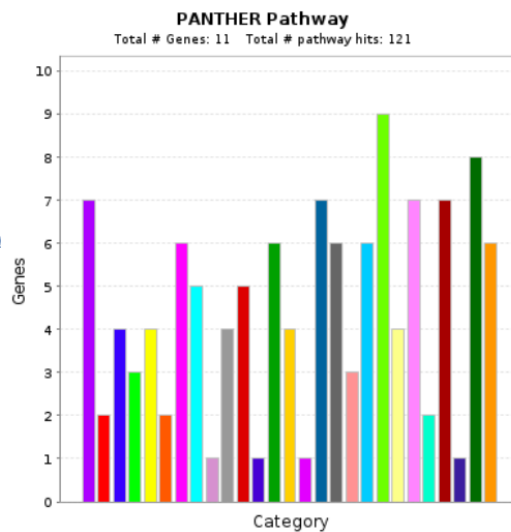


Figure 2. This figure shows the molecular pathway in which *PIK3CA* interacting proteins are involved.

PIK3CA: Phosphatidylinositol 3-kinase

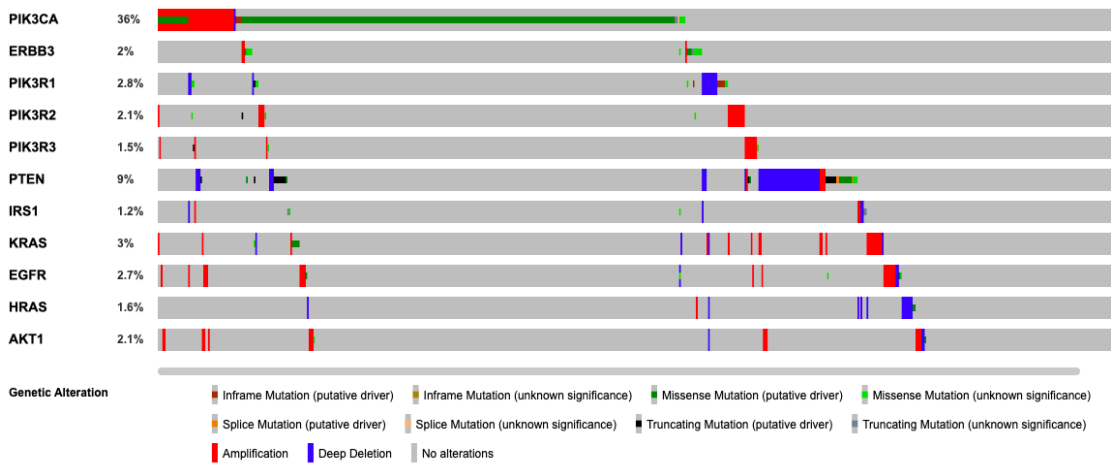


Figure 3. This figure depicts the OncoPrint data demonstrating genetic alterations in *PIK3CA* interacting genes in 963 patients of the Breast Invasive Carcinoma Dataset (TCGA, Firehose Legacy).

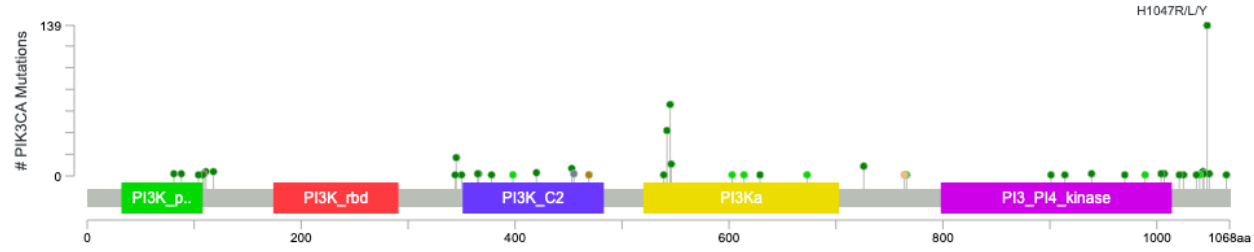


Figure 4. This figure depicts the Lollipop plot demonstrating the position of mutations in the *PIK3CA* gene as observed with the Breast Invasive Carcinoma Database [TCGA, Firehose Legacy].

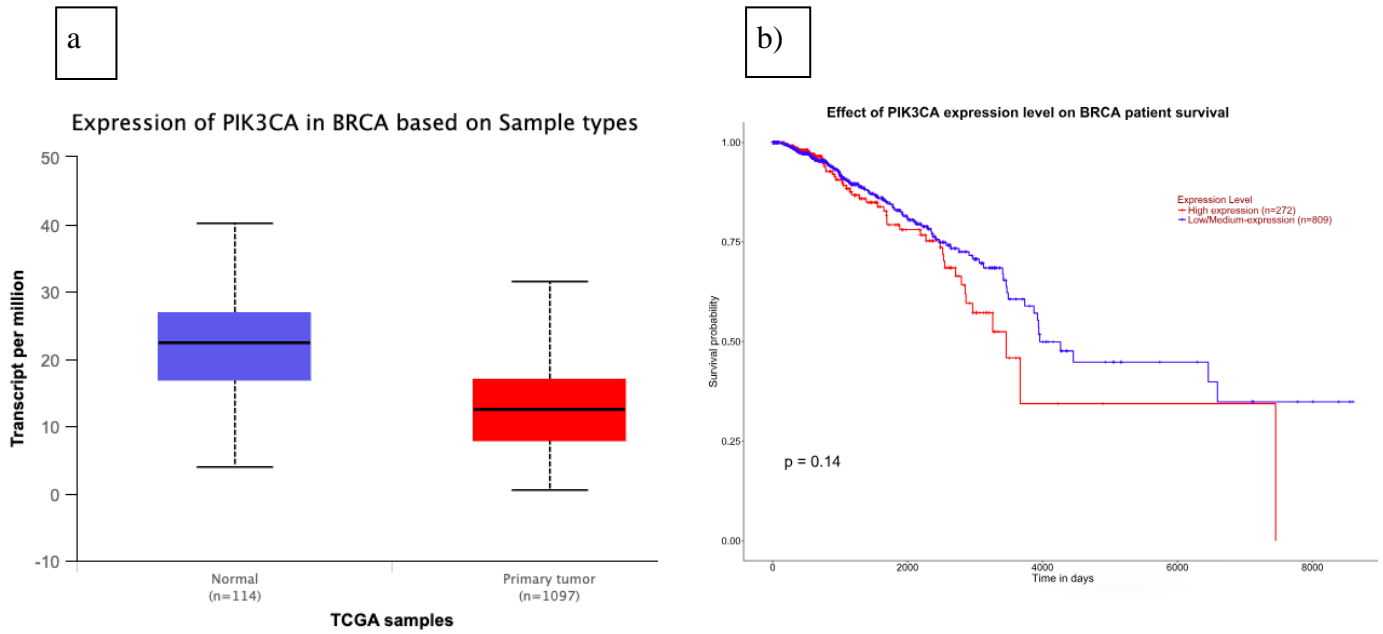


Figure 5. This figure shows (a) Box whisker plot demonstrating the expression profile of the *PIK3CA* gene in the Breast Invasive Carcinoma Dataset. A significant downregulation in the gene expression was observed between the normal and primary tissues ($P = 1.62 \times 10^{-12}$). (b) The Kaplan-Meier Survival analysis revealed no significant association between the gene expression levels and survival probability ($P = 0.76$). (A P -value less than 0.05 was considered significant).