IJBPAS, June, 2022, 11(6): 2871-2882

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ACTIVATION OF ONCOGENIC AND NON-ONCOGENIC INTERLINKED PATHWAYS WHEN PTEN GENE IS MUTATED IN BREAST INVASIVE CARCINOMA OF PAN-CANCER ATLAS METADATA

PATADIA HK[1, 2, 5], UPADHAYAY D[3], AGHERA R[1] AND GANGAWANE A[4*]

1: PhD Scholar, Faculty of Applied Sciences, Parul University, Vadodara, Gujarat, India.

2: PhD Scholar, Department of Microbiology, Parul Institute of Applied Sciences, Parul University, Vadodara, Gujarat, India.

3: Assistant Professor, Parul Institute of Applied Sciences, Parul University, Post Limda, Waghodia, Gujarat, 391760

4: Professor, Faculty of Applied Sciences, Parul University, Vadodara, Gujarat, India.

5: Department of Paramedical and Health Sciences, Faculty of Medicine, Parul University, Vadodara, Gujarat, India

[*]**Corresponding Author: Dr. Ajit Gangawane:** ajit.gangawane@paruluniversity.ac.in

Received 18th Feb. 2022; Revised 19th March. 2022; Accepted 9th April 2022; Available online 1st June 2022

https://doi.org/10.31032/IJBPAS/2022/11.6.6854

ABSTRACT

Background: PTEN is one of the foremost typically mutated human growth suppressor cistrons involved at intervals the expansion and survival of cells, likewise as within the suppression of tumor formation. Loss of PTEN activity, either at the super molecule or genomic level, has been involving many primary and pathologic process malignancies moreover as malignant neoplastic disease and pathological process malignancies as well as breast cancer. Methods: Data were taken from cBioportal platform for cancer genomics therein we chose study of Breast Invasive malignant neoplastic disease of TCGA Pan-cancer Atlas, from that study we identified 227 genes of RAS-signaling pathway and downloaded mutation data and template RNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2) incorporates 918 samples of primary

carcinoma samples within which fifty-seven samples showing PTEN mutation. Then we make data matrix file in line with iDEP.91 portal guidelines. Results: Heatmap showed clusters of genes across the samples explains the regulation of genes. K-means clustering identified five clusters based on Elbow plot in KEGG Enrichment. Cluster E showed the most variety factors enrichment in carcinoma pathways regulation. Conclusion: Once the PTEN factor is mutated in carcinoma, it causes several oncogenes and non-oncogenes to be triggered and suppressed. RAS-signaling pathway activated tumor microenvironments which were interlinked with many pathways for supporting breast cancer tumor progression, cell growth and proliferation.

Keywords: PTEN gene, Cancer, Oncogene, Breast cancer, India

INTRODUCTION:

The phosphatase and tensin homolog deleted on chromosome ten (PTEN) is one in every of the foremost often mutated human tumor suppressor genes involved in cell growth and survival and suppressing tumor formation. Loss of PTEN activity, either at the macromolecule or genomic level, has been regarding many primary and metastatic malignancies together with carcinoma **[1]**. The PTEN protein is wide notable for its role in catalyzing the dephosphorylation of phosphatidylinositol (3,4,5)-triphosphate (PIP3), a vital intracellular second messenger, with bigger alacrity, lowering its level at intervals the cell **[2]**. The neoplasm suppressor operate of PTEN relies on its lipid enzyme activity and therefore the loss-of-function of the phosphatase chemical process domain is often related to oncogenic PTEN mutations. Several studies offer proof of PTEN loss in activation and liberation of signal transduction pathways like RAS-Signaling pathway, Rap1-Signaling pathway, mTOR signaling pathway and PI3K/Atk-Signaling pathway **[3–5]**. PTEN encodes a twin supermolecule and lipid phosphatase 1,2 that the flexibility to negatively regulate the phosphatidylinositol-3'-kinase (PI3K)/Akt pathway is central to its neoplasm suppressor operate. RAS and PTEN directly participate within the regulation of a posh communication network that affects cellular functions unremarkably deregulated throughout tumorigenesis. Activation of Ras through oncogenic mutations that happens often during DMBA-TPA evoked skin carcinogenesis stimulates MAPK and Akt signaling. The Akt pathway is in restraint of Pten, which could be a target for loss-of-function alterations in cancer. Upregulation of Akt activity could result in suppression of MAPK through inhibition of the Raf kinase.

Inhibition of downstream targets of Akt, equivalent to mTor, can lead to an improvement of Akt activity because of the loss of feedback restrictive mechanisms projected to occur through inhibition of irs proteins (insulin receptor substrates) by activated p7056K. Once PTEN is mutated the overexpression of ErbB2 ends up in its activation through autophosphorylation (P) **[6]**. As a result, Src enzyme and phosphatidylinositol 3' kinase (PI3K), with its regulative subunits p85 and p110, are recruited to the receptor and unbroken in their active state. The activation of PI3K leads successively to the activation of the proto-oncogenic signal pathway consisting of Akt and therefore the class target of rapamycin (mTOR). When active, the Src protein will inactivate PTEN through the phosphorylation of its C-terminal end. This trigger the assembly of elevated levels of phosphatidylinositol 3,4,5-triphosphate (PIP3), any potentiating the activation of PI3K. On binding to the ErbB2 receptor, trastuzumab causes the dissociation of the receptor from Src and its inactivation through unknown mechanisms. PTEN therefore becomes unengaged to antagonize the activation of the PI3K–AKT–mTOR signaling pathway through the dephosphorylation of PIP3. Trastuzumab may well be combined with medicine adore sirolimus (rapamycin) and its analogues, everolimus (RAD001) and CCI-779, that inhibit mTOR, to dam this vital signaling pathway at 2 totally different points. A partial or total deficiency of PTEN might account for resistance to trastuzumab. The Cancer order Atlas (TCGA) could be a vast information supply for cancer genomics. The Cancer order Atlas (TCGA) is a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of cancer through the appliance of genome analysis technologies, as well as large-scale genome sequencing **[7]**. TCGA includes data from over thirty-three cancer types, with RNA-Seq, DNA-Seq, Copy Number, Methylation, Expression array (Agilent), and macromolecule array (RPPA) data. Knowledge of TCGA is out there on several cancer data portals like GDC portal (https://portal.gdc.cancer.gov/), COSMIC (https://cancer.sanger.ac.uk/cosmic), cBioportal (https://www.cbioportal.org/) and plenty of that helps in data mining, visualisation and analysis of information across completely different cancer types. Our study data is collected from cBioportal (https://www.cbioportal.org/) this is often a platform for cancer genomic data is intended for data analysis and visualization of

designated cancer datasets. Any analysis of data is finished in online web-portal iDEP.91 (http://bioinformatics.sdstate.edu/idep/). This is often a platform for data analysis which have feature of pre-processing of data, exploratory data analysis, heatmaps, k-means clustering, hierarchal clustering, Principal component analysis, pathway analysis. iDEP (integrated Differential Expression and Pathway analysis) seamlessly connect sixty-three R/Bioconductor packages, two web services, and comprehensive annotation and pathway **[8]**. This research aimed at understanding about the PTEN factor mutation in carcinoma and its role in several oncogenes and non-oncogenes.

METHODS

Study population

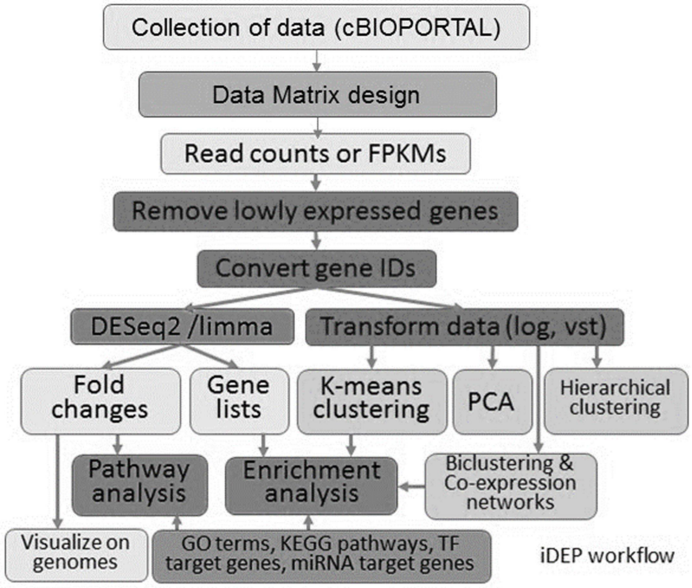

Figure 1: Road map of analysis

Data collection

Collection of data began from cBioportal platform for cancer genomics. Within this platform, we chose the study of Breast Invasive malignant neoplastic disease of TCGA Pan-cancer Atlas. From this study we identified 227 genes of RAS-signaling pathway and downloaded mutation data and template RNA Expression and RSEM (Batch normalized from Illumina HiSeq_RNASeqV2) incorporated 918 samples of primary carcinoma samples within which fifty-seven samples showed PTEN mutation. Later, we prepared data matrix file in line with iDEP.91 portal guidelines.

Statistical analysis

Data were reported by using Principal Component Analysis.

RESULTS

Heatmap in the below **Figure 2** was generated after pre-processing and filtering of low expressed genes. Heatmap showed clusters of genes across the samples explains the regulation of genes. Our study hand-picked the high expressing genes for any process of analysis.

Additionally, this study performed k-means agglomeration of data. Below **Figure 3** ascertained differential expression of genes enrichment in KEGG pathways. K-means clustering in below **Figure 3** identified five clusters based on Elbow plot in KEGG Enrichment. Cluster E is showing most variety factors enrichment in carcinoma pathways regulation. From K-means maximum number of genes are enriched in Ras-signaling pathway.

Principal Component Analysis (PCA) in 2 dimensions was performed to examine the variance of the gene in samples we performed as shown in below **Figure 4**. Principal Component Analysis performed showed about 15% PC1 and 12% PC2 variance among Wildtype and Mutated Samples of PTEN gene.

This study performed Differential factor (DEGs) by taking FDR cut-off 0.5 and minimum fold modification one as shown in **Figure 5**. This study checked the differential expression of genes of variable genes using limma package and located eighteen up-regulated and 18 down-regulated gens by taking 1-fold modification

The differentially expressed genes in KEGG pathway were tabulated in below **Table 1**. Pathway analysis of Differentially Expressed genes is completed with technique PGSEA w/all samples with KEGG pathway enrichment analysis taking pathway FDR cut-off 0.5 to examine and analysis expression of genes in pathways.

Our pathway clustering showed that the activation and suppression of genes were interlinked with several pathways this interlink in the pathway support for breast tumorigenesis as shown in below **Figure 6**.

RAS-signaling pathway in KEGG showed red colored gene as showing overexpression and green color gene showed suppressed expression of genes in regulation of pathway. Our analysis showed that activation of RAS gene supermolecule was incredibly high once PTEN gene was mutated. Activation of RAS gene led to PI3K, RAC, RGL overexpression that in turn led to development of neoplastic cell resistance of growth activity **[9]**. PI3K/ATK had potential role in tumorigenesis, proliferation, growth, apoptosis, invasion, metastasis, immune microenvironment and drug resistance of cancer cells. This pathway activation was

because of activation of over expression genes like ETS2 RGL1 PIK3CD RAC1 RAC2 RALA RALB RASAL3 RASSF5 PIK3R3 CDKN1A NFE2L2 **[10]**. This activation was result of RAS and PTEN to directly participate within the regulation of a posh signaling network that affected cellular functions ordinarily deregulated throughout tumorigenesis. Activated RAS activity once PTEN was mutated is shown in below **Figure 7** which show tumorigenesis in breast cancer.

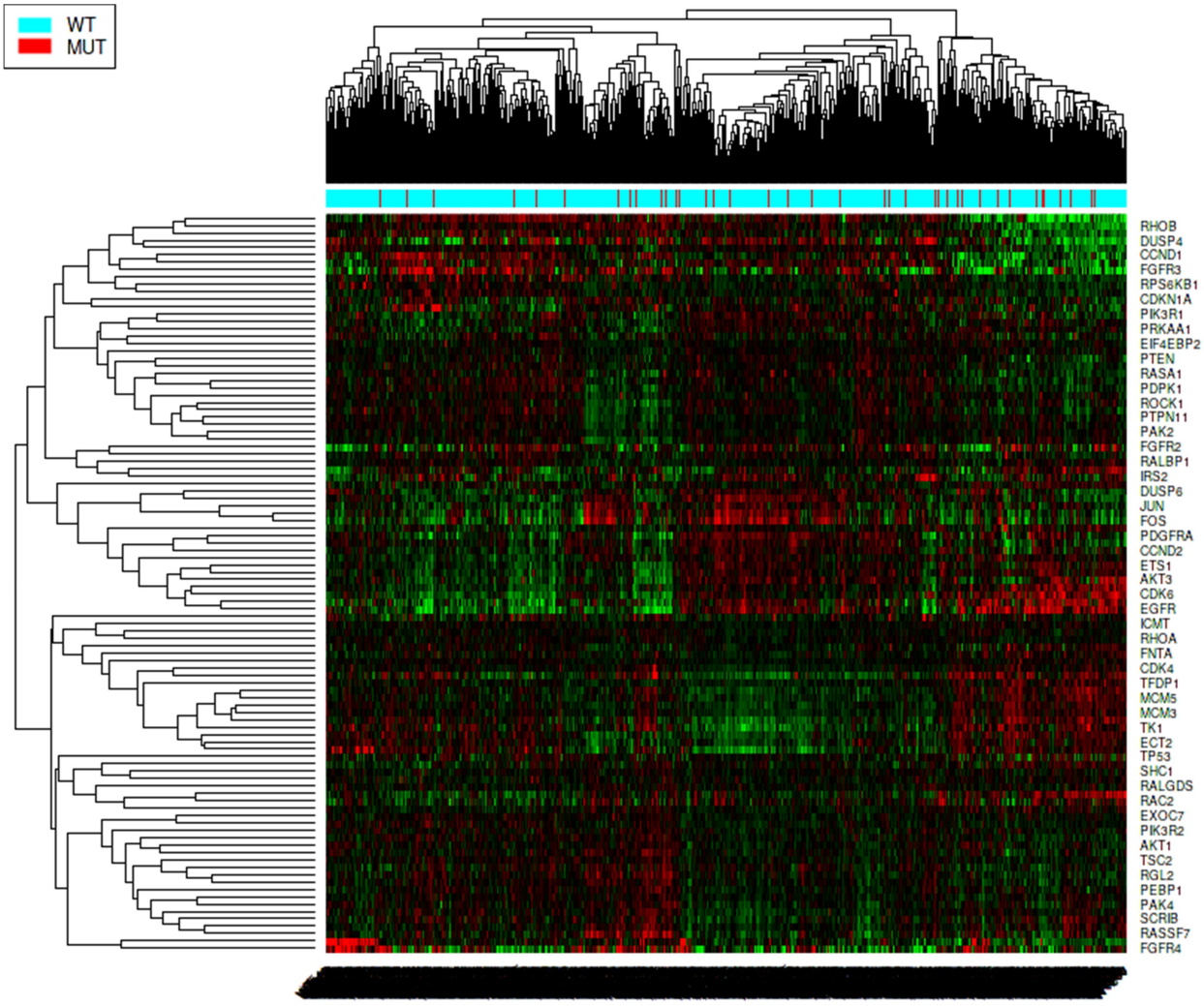

Figure 2: Heatmap of sample vs genes showing their expression when PTEN gene is mutating and normal. Hierarchical clustering is done within genes and samples to check internal relation of genes and samples

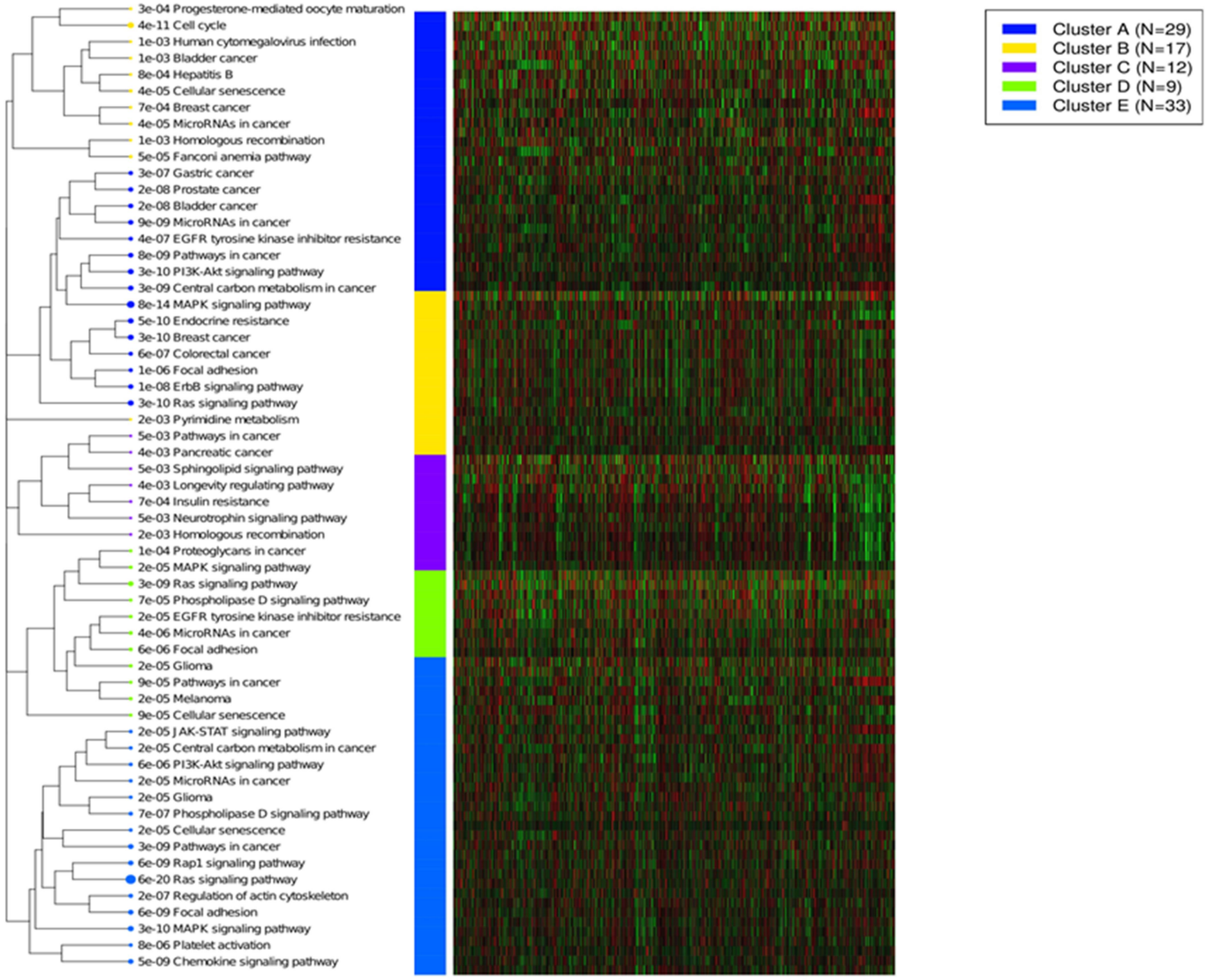

Figure 3: Kmeans heatmap with pathway enrichment in KEGG in which cluster E is showing maximum enrichment of genes in breast cancer pathway

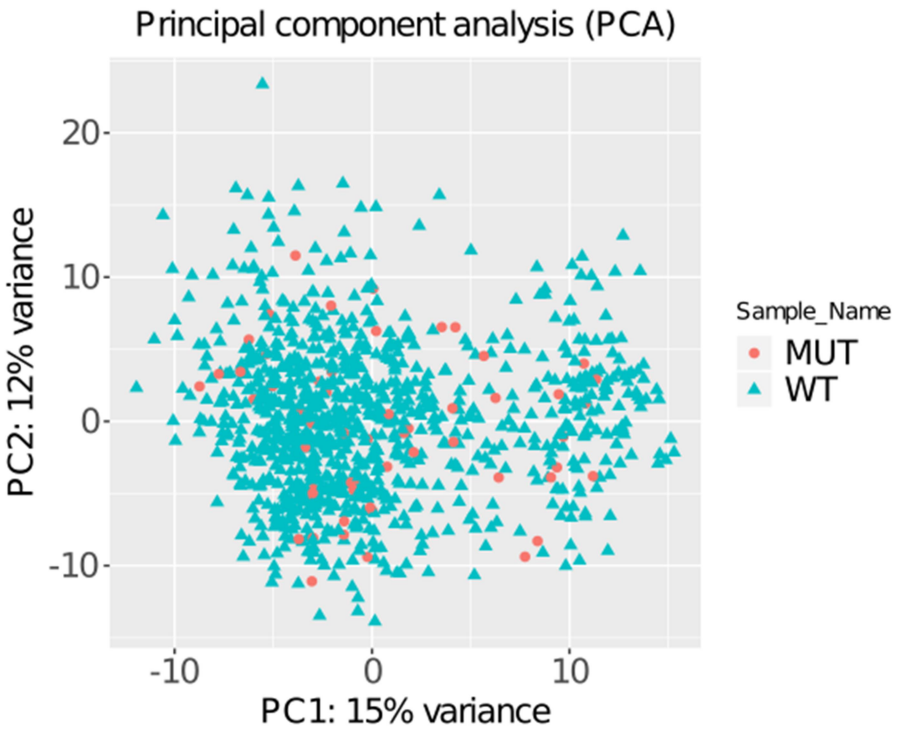

Figure 4: Principal Component Analysis (PCA) is showing variance in sample inPC1 and PC2 which is showing very low variance in gene expression in sample when PTEN is MUT and WT

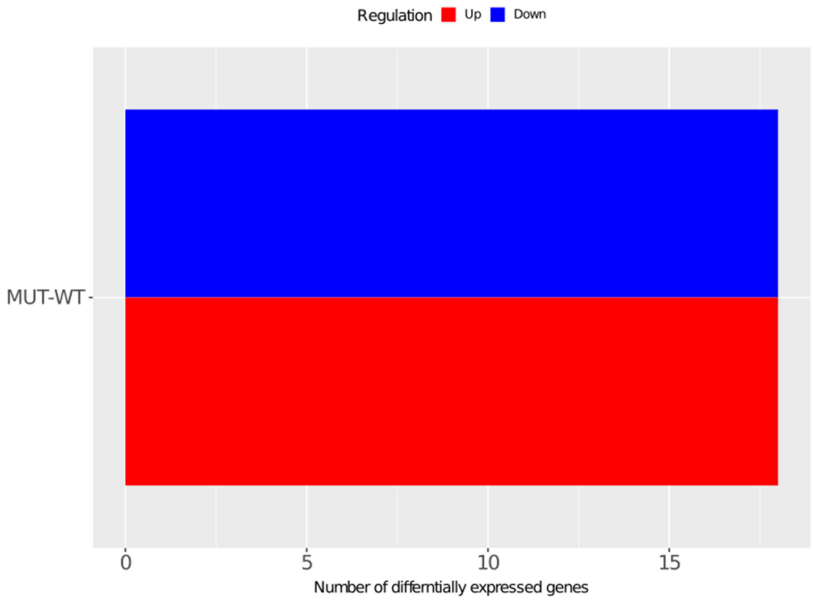

Figure 5: Differential Expression of genes (DEGs) in limma-voom taking FDR cut-off 0.5 showing 18 up-regulated and 18 down-regulated genes against mutation vs normal samples

Table 1: KEGG pathway enrichment with differentially expressed genes (DEGs) which shows down-regulation of PI3k-Akt signaling pathway, AMPK signaling pathway and MTOR signaling pathway. Up-regulation of RAS-signaling pathway, RAP1 signaling pathway and Pathways in cancer

Direction	adj.Pval	nGenes	Pathways
Down regulated	1.50E-10	7	FoxO signaling pathway
	1.50E-10	7	Insulin signaling pathway
	1.50E-09	8	PI3K-Akt signaling pathway
	3.80E-09	6	AMPK signaling pathway
	4.50E-09	6	Autophagy
	6.10E-09	5	Endometrial cancer
	7.00E-09	6	MTOR signaling pathway
	7.00E-09	5	Longevity regulating pathway
	7.00E-09	6	MicroRNAs in cancer
	7.30E-08	5	Insulin resistance
	7.70E-07	4	Non-small cell lung cancer
	1.70E-05	3	Bladder cancer
	1.70E-05	4	Breast cancer
Up regulated	3.90E-14	10	Ras signaling pathway
	4.70E-12	7	Pancreatic cancer
	4.90E-11	10	Pathways in cancer
	1.00E-09	6	Colorectal cancer
	2.50E-09	7	Rap1 signaling pathway
	3.50E-09	6	Leukocyte transendothelial migration
	2.30E-08	5	Fc epsilon RI signaling pathway
	4.90E-08	6	Chemokine signaling pathway
	4.90E-08	6	Kaposi sarcoma-associated herpesvirus infection
	1.10E-07	6	Human cytomegalovirus infection
	4.20E-07	5	Fluid shear stress and atherosclerosis
	5.50E-07	5	Phospholipase D signaling pathway

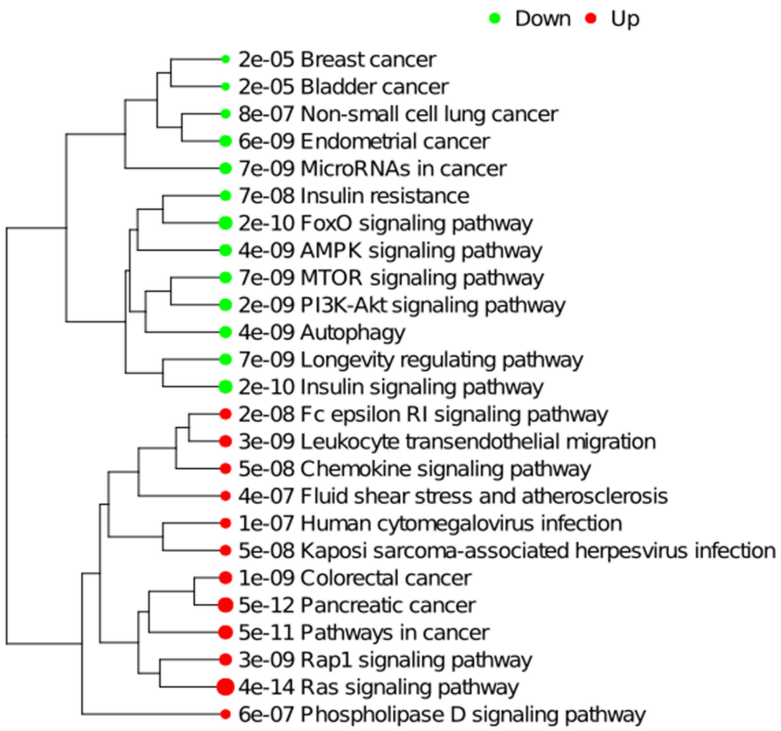

Figure 6: Clustering of pathway based on Up and Down regulation in KEGG Enrichment analysis

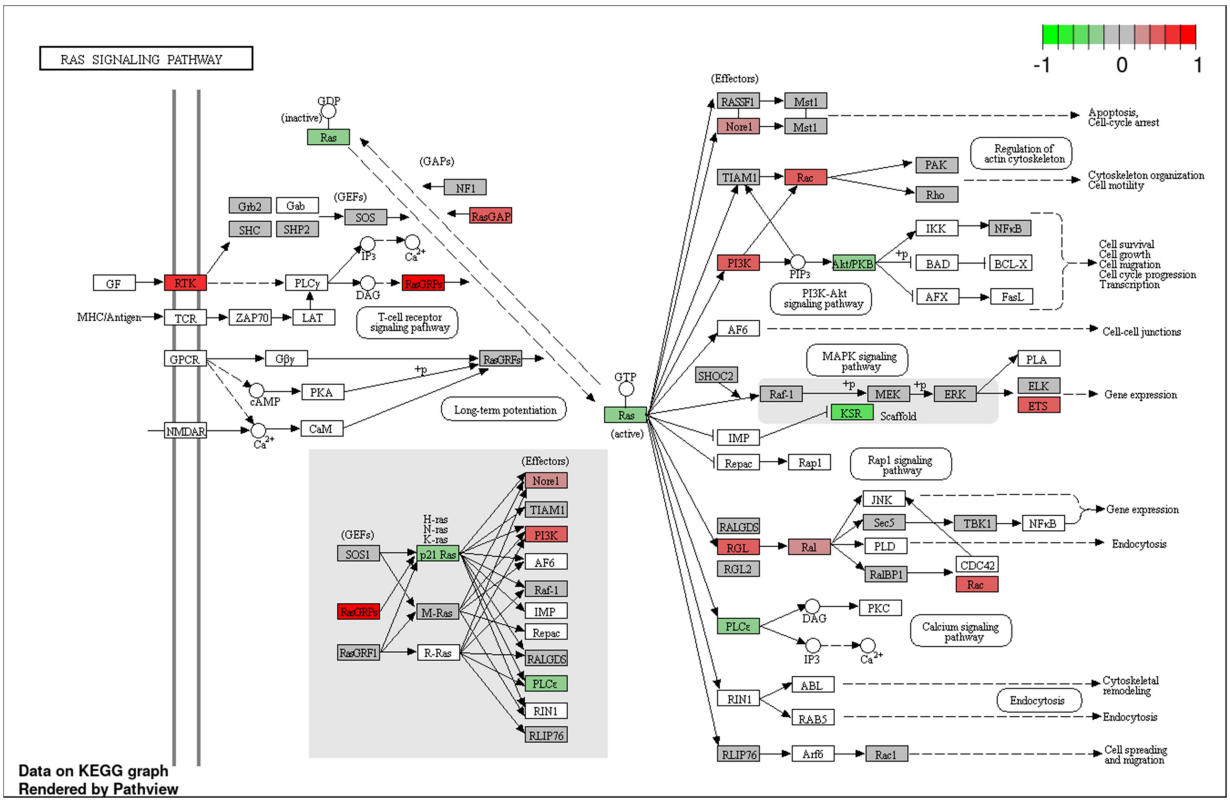

Figure 7: Upregulation of RAS-signaling pathway in KEGG

DISCUSSION

The PTEN neoplasm suppressor factor, situated on chromosome 10q23, is one among the foremost often mutated gene in cancer **[11]**. Its germline mutations cause Cowden syndrome that is characterised by an enhanced risk for the event of benign and malignant tumors. Irregular PTEN mutations are discovered frequently in a form of human neoplasm, as well as breast cancer.

Loss of operate of the PTEN tumor suppressor can cause essential activation of the PI3K/Akt/mTOR pathway. The PI3K/Akt/mTOR pathway has been shown to play important role in cancer by regulation apoptosis, neoangiogenesis and proliferation and has become a crucial target for antitumor drug development **[12]**. Development of therapeutic ways for PTEN and RAS alteration could be a challenge for several researchers. Pathways involving PTEN and RAS represent a posh intertwining signaling network with multiple purpose of interaction and varied feedback mechanism. Neoplasm with complete loss of PTEN and essential high-level activation of AKT could also be refractory to inhibition of RAS signaling through the utilization of inhibitors.

Our data analysis proves that RAS-signaling pathway makes tumor microenvironment

with one another because of that resistance in neoplastic cell growth activity is found. Frequent activation of this pathway in cancer is failure response of drug that resist antitumor activity. This study concludes that once the PTEN factor is mutated in carcinoma, it causes several oncogenes and non-oncogenes to be triggered and suppressed. RAS-signaling pathway activated tumor microenvironments which were interlinked with many pathways for supporting breast cancer tumor progression, cell growth and proliferation. Therefore, we suggest that by inhibiting RAS pathway, resistance in cancer therapies can also be controlled.

Acknowledgments

We thank all the contributors who contributed for technical inputs in this study.

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