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ORIGINAL ARTICLE **OPEN ACCESS**

Activation of Oncogenic and Non-Oncogenic interlinked Pathways when PTEN gene is mutated in Breast Invasive carcinoma of Pan-cancer Atlas Metadata

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ABSTRACT
PTEN is one of the most frequently mutated human tumour suppressor genes, implicated in cell growth and survival and suppressing tumour formation. Loss of PTEN activity which can be seen either at the protein or genomic level which can relate to many primary and metastatic malignancies including breast cancer. In our metadata study of TCGA BRCA (Pan-cancer Atlas) from cBioportal (https://www.cbioportal.org/) investigates mutation of PTEN gene in 57 samples against non-mutated samples and further investigates its effect of mutation in activation of signal transduction pathway. PTEN mutation leads to activation in many oncogenic signalling pathways and our study is focused on to find out non-oncogenic gene which up-regulate and down-regulate signal transduction pathway which is playing supportive role in breast tumorigenesis.
***Keywords**: PTEN, Oncogene, tumour suppressor genes*

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INTRODUCTION

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is one altogether the foremost frequently mutated human tumor suppressor genes, implicated in cell growth and survival and suppressing tumor formation [1]. The PTEN protein is widely known for its role in catalyzing the dephosphorylation of phosphatidylinositol (3,4,5)-triphosphate (PIP3), a vital intracellular second messenger, with greater alacrity, lowering its level within the cell [2]. The tumor suppressor function of PTEN relies on its phospholipid phosphatase activity and thus the loss-of-function of the phosphatase catalytic domain is typically associated with oncogenic PTEN mutations [2, 3, 4]. Many studies give evidence of PTEN loss in activation and deregulation of signal transduction pathways like RAS-Signalling pathway, Rap1-Signalling pathway, mTOR signalling pathway and PI3K/Atk-Signalling pathway. Pten encodes a dual protein and lipid phosphatase1,2 that the facility to negatively regulate the phosphatidylinositol-3'-kinase (PI3K)/Akt pathway is central to its tumor suppressor function [3]. Ras and Pten directly participate within the regulation of a flowery signalling network that affects cellular functions commonly deregulated during tumorigenesis. DMBA-TPA induced skin carcinogenesis frequently results in activation of Ras through oncogenic mutation, stimulates MAPK and Akt signalling. The Akt pathway is in check of Pten, which might be a target for loss-of-function alterations in cancer. Suppression of MAPK through inhibition of the Raf kinase can be seen by Upregulation of Akt activity. Inhibition of downstream targets of Akt, like mTor, can cause an enhancement of Akt activity because of the loss of feedback inhibitory mechanisms proposed to occur through inhibition of IRS proteins (insulin receptor substrates) by activated p7056K [3-8]. When PTEN is mutated the overexpression of ErbB2 winds up in its activation through autophosphorylation (P). As a result, Src kinase and phosphatidylinositol 3' kinase (PI3K) along with their subunits, are activated when attached to the receptor. The activation of PI3K leads successively to the activation of the proto-oncogenic signalling pathway consisting of Akt and thus the mammalian target of rapamycin (mTOR). Nagata and colleagues [1, 2, 4] show that when active, Src can inactivate PTEN through the phosphorylation of its C-terminal

end. This triggers the assembly of elevated levels of phosphatidylinositol 3,4,5-triphosphate (PIP3), further potentiating the activation of PI3K. Trastuzumab causes the dissociation of the receptor from Src and its inactivation through unknown mechanisms, On binding to the ErbB2 receptor. PTEN thus becomes free to antagonize the activation of the PI3K–AKT–mTOR signalling pathway through the dephosphorylation of PIP3. Trastuzumab may be combined with drugs like sirolimus (rapamycin) and its analogues, everolimus (RAD001) and CCI-779, which inhibit mTOR, to dam this critical signalling pathway at two different points. Nagata et al. show that a partial or total deficiency of PTEN may account for resistance to trastuzumab [1, 4].

The Cancer Genome Atlas (TCGA) might be an enormous data source for cancer genomics. The Cancer Genome Atlas (TCGA) can be a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of cancer through the appliance of genome analysis technologies, including large-scale genome sequencing. TCGA includes data from over 33 cancer types, with RNA-Seq, DNA-Seq, Copy Number, Methylation, and Expression array (Agilent), and protein array (RPPA) data. Data of TCGA is obtainable on many cancer data portals like GDC portal, COSMIC, cBioportal and much of more which helps in processing, visualization and analysis of information across different cancer types.

Our study data is collected from cBioportal this can be often a platform for cancer genomic data is supposed for data analysis and visualization of selected cancer datasets. Further analysis of information is completed in online web-portal iDEP.91 this may be a platform for data analysis which have feature of pre-processing of data, Exploratory data analysis, heatmaps, k-means clustering, hierarchical clustering, Principal component analysis, pathway analysis in iDEP (integrated Differential Expression and Pathway analysis) [5, 13-15].

MATERIAL AND METHODS [9-12]

Collection of Data

We have collected our data from cBioportal platform for cancer genomics therein we select study of Breast Invasive Carcinoma of TCGA Pan-cancer Atlas, from that study we quired 227 genes of RAS-signalling pathway and downloaded mutation data and mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2) comprises 918 samples of primary carcinoma samples within which 57 samples showing PTEN mutation. Then we make data matrix file per iDEP.91 portal guidelines.

Statistical Analysis

We have done pre-processing of information in iDEP.91 to strain noisy data from our data matrix with Exploratory Data analysis to achieve knowledge of information as shown in Fig.2.

Then we remove genes with low expression. Further we performed k-means clustering of knowledge.

To test differential expression of genes enrichment in KEGG pathways as shown in Fig.3.

To check variance of the gene in samples we performed Principal component analysis (PCA) in two dimensions as shown in Fig.4.

We performed Differential organic phenomenon (DEGs) by taking FDR cut-off 0.5 and minimum fold change 1 as shown in Fig.5.

Then we check our differentially expressed genes in KEGG pathway as shown in Table 1.

Pathway analysis of Differentially Expressed genes is completed with method PGSEA w/all samples with KEGG pathway enrichment analysis taking pathway FDR cut-off 0.5 to visualise and analysis expression of genes in pathways.

Figure 2 outlines the iDEP workflow. Expression matrix is first filtered, transformed and converted to Ensemble gene IDs, which are used internally to spot genes. The pre-processed data is then used for EDA, with methods like K-means clustering, hierarchical clustering, principal component analysis (PCA), and t-SNE. Gene clusters identified by K-means are analyzed by enrichment analysis supported an outsized gene annotation and pathway database in Figure 3. Figure 5 shows the identification of DEGs is completed with either the limma packages. This is often also followed by enrichment analysis on the DEGs. The fold-change values are then employed in pathway analysis using several methods. Pre-processing of information is completed to get rid of lowely expressed genes. EDA enables the users to explore variations and patterns within the dataset as a full. The most methods include hierarchical clustering with heatmap, k-means clustering, and PCA. Enrichment analysis of genes derived from k-means clustering is conducted to realize insights into the functions of co-expressed genes. Initial attempts of pathway analysis are dispensed using the PCA loadings on each gene. This will tell us the biological processes underlying each direction of expression change defined by the principal components. PCA allows us to project samples into 2-D space. To run pathway analysis with the PGSEA package, we treated the PCA loadings onto each of the genes as expression data. For every pathway, this runs the PAGE algorithm which performs one-sample t-test on each gene set. The adjusted P-values are wont to rank the pathways for every of the primary 5 principal components. The pathways are labelled with FDR first,

followed by the principal components (PC1, PC2 and then on) mentioned in Figure 4. The PGSEA package implements the Parametric Analysis of Gene Set Enrichment (PAGE) algorithm to display the activities of pathways in individual samples in terms of Z scores, which characterize what quantity the mean of the fold-changes for genes during a certain pathway deviates from the mean observed altogether the genes. We revise and modified the PGSEA code by adding an analysis of variance (ANOVA) on the basis of Z scores among sample groups. Also, after cut-off with FDR, pathways are ranked by the quality deviation. This modification gives meaningful information i.e. intuitive display of differentially regulated pathways among sample groups.

RESULT

Heatmap in figure 2 is generated after pre-processing and filtering of low expressed genes. Heatmap is showing clusters of genes across the samples explains the regulation of genes. we selected top expressing genes for further processing of analysis. K-means clustering in figure 3 identifies 5 clusters based on Elbow plot in KEGG Enrichment. Cluster E is showing maximum number genes enrichment in breast cancer pathways regulation. From K-means maximum number of genes are enriched in Ras-signalling pathway. To check Variance among genes in breast cancer pathways principle component analysis is performed which shows 15% PC1 and 12% PC2 variance among Wildtype and Mutated Samples of PTEN gene shown in Figure 4. We checked differential expression of genes of variable genes using limma package and found 18 up-regulated and 18 down-regulated gens by taking 1-fold change as shown in Figure 5. These genes are mapped in pathways to check regulation of pathways with differentially expressed genes as shown in table 1. Pathways clustering in Figure 6 shows clusters among pathways Ras-signalling pathway is sharing it node to Rap1-signalling pathway this two cluster is sharing their nodes with pathways in cancer all are responsible for Breast cancer. Pathway enrichment using PGSEA w/all samples in KEGG pathways to shows regulation of genes in the pathway. Ras-signalling pathway in KEGG showing red coloured gene is showing overexpression and green colour gene is showing supressed expression of genes in regulation of pathway. Our evaluation on basis of analysis and experiments done earlier in other studies identified activation of RAS gene protein is very high when PTEN gene is mutated. Activation of RAS gene results in PI3K, RAC, RGL overexpression which leads to development of cancer cell resistance of antitumour activity. PI3K/ATK has potential role in tumorigenesis, proliferation, growth, apoptosis, invasion, metastasis, immune microenvironment and drug resistance of cancer cells. This pathway activation is due to activation of over expression this genes ETS2 RGL1 PIK3CD RAC1 RAC2 RALA RALB RASAL3 RASSF5 PIK3R3 CDKN1A NFE2L2. This activation is because RAS and PTEN is directly participate in the regulation of a complex signalling network that affects cellular functions commonly deregulated during tumorigenesis. Activated RAS activity when PTEN is mutated shown in Fig.7, show tumorigenesis in breast cancer. Evidence of down regulation of AMPK shows increased potential in breast cancer due to alteration in energy supply, can regulate protein and lipid metabolism. Over expression of RAP1 is linked with increased in breast cancer metastasis as cell migration is early requirement for tumour metastasis as shown Fig.8. Our pathway clustering shows that activation and suppression of genes are interlinked with many pathways this interlinks in pathway support breast tumorigenesis as shown Fig.6

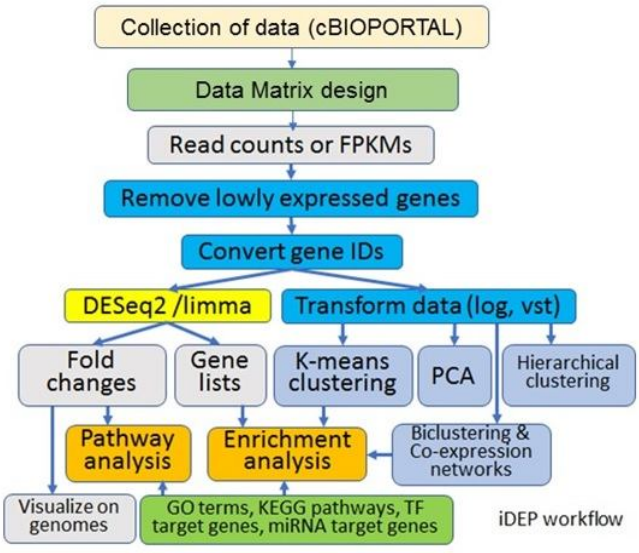

Figure 1. Road map of analysis

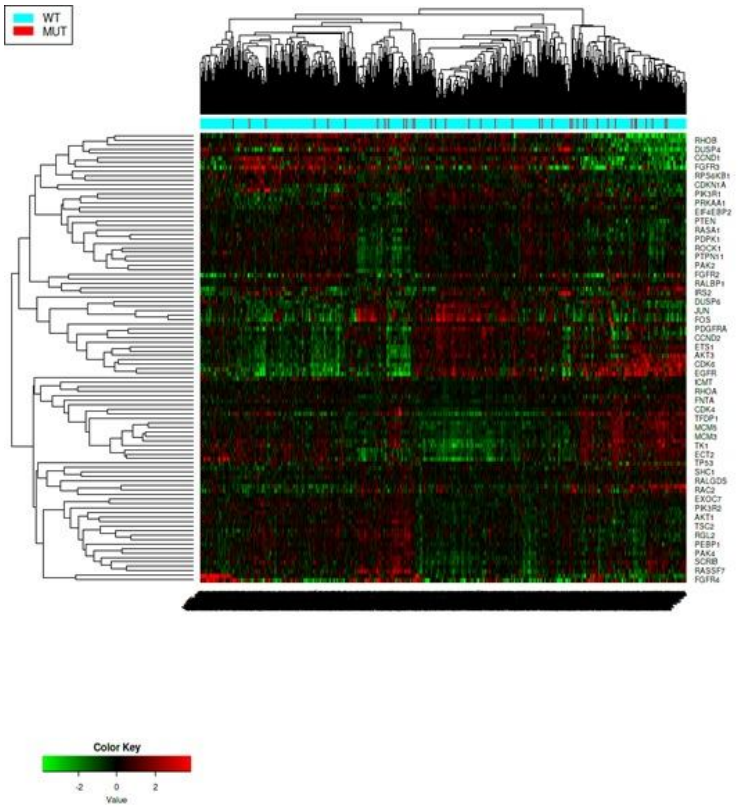

Fig.2. Heatmap of sample vs genes showing their expression when PTEN gene is mutating and normal. Herarchial clustering is done within genes and samples to check internal relation of genes and samples.

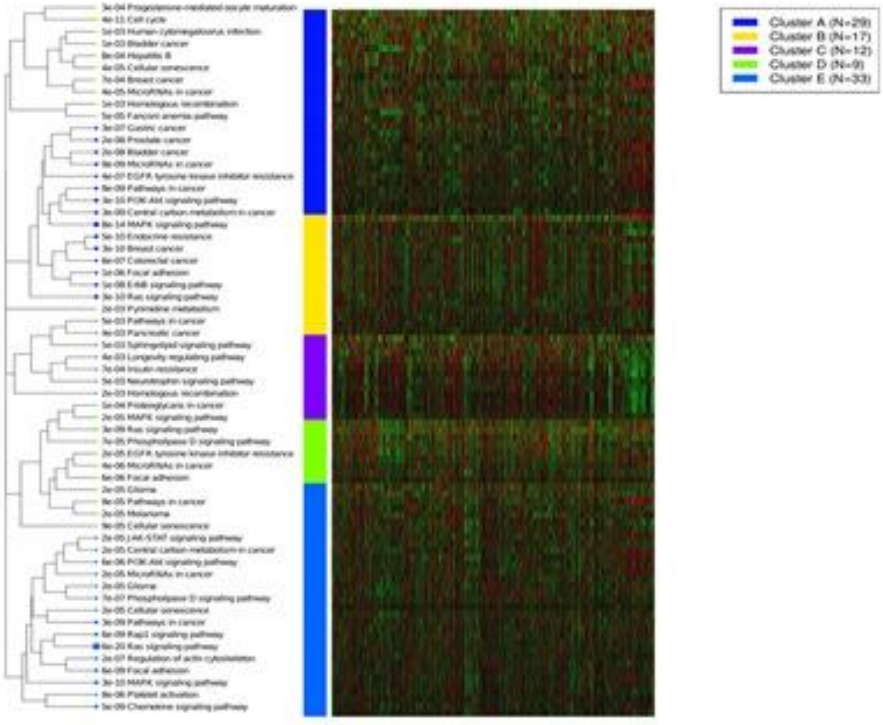

Fig.3. Kmeans heatmap with pathway enrichment in KEGG in which cluster E is showing maximum enrichment of genes in breast cancer pathway.

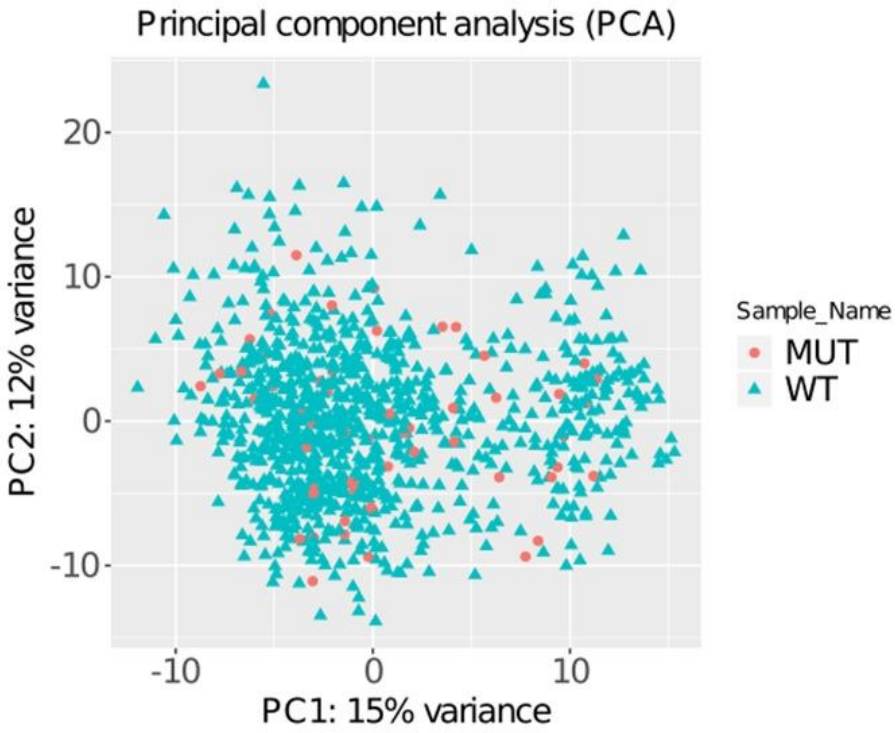

Fig.4. Principle component analysis (PCA) is showing variance in sample inPC1 and PC2 which is showing very low variance in gene expression in sample when PTEN is MUT and WT.

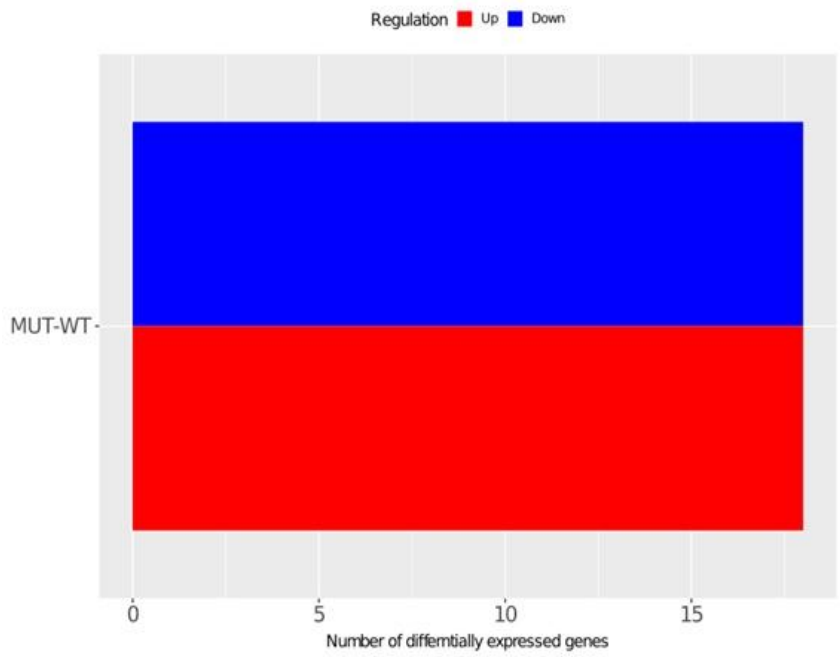

Fig.5. Differential Expression of genes (DEGs) in limma-voom taking FDR cut-off 0.5 showing 18 up-regulated and 18 down-regulated genes against mutation vs normal samples.

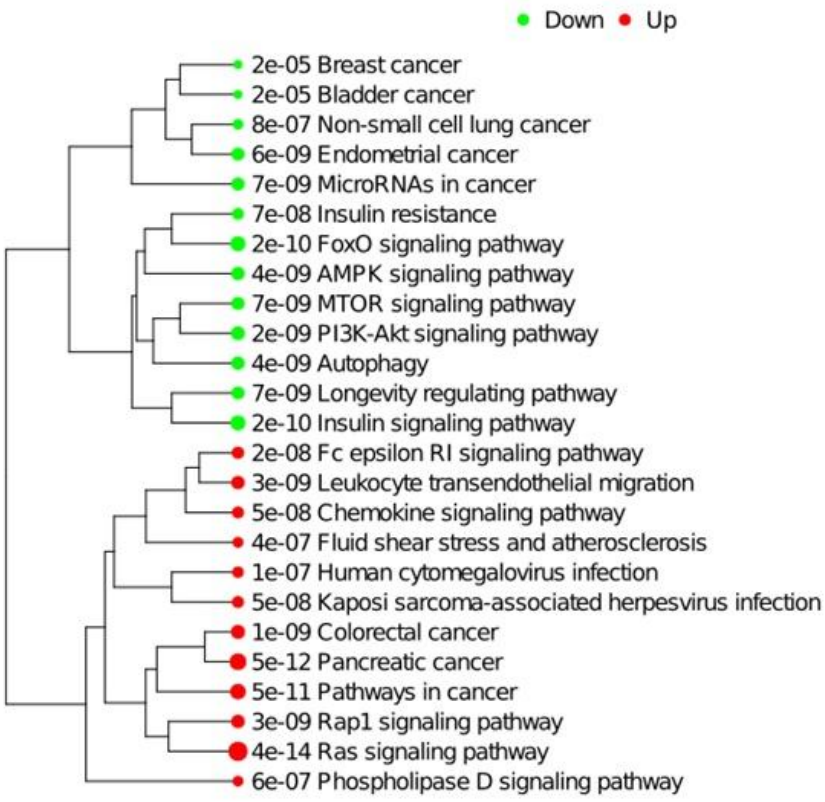

Fig.6: Clustering of pathway based on Up and Down regulation in KEGG Enrichment analysis.

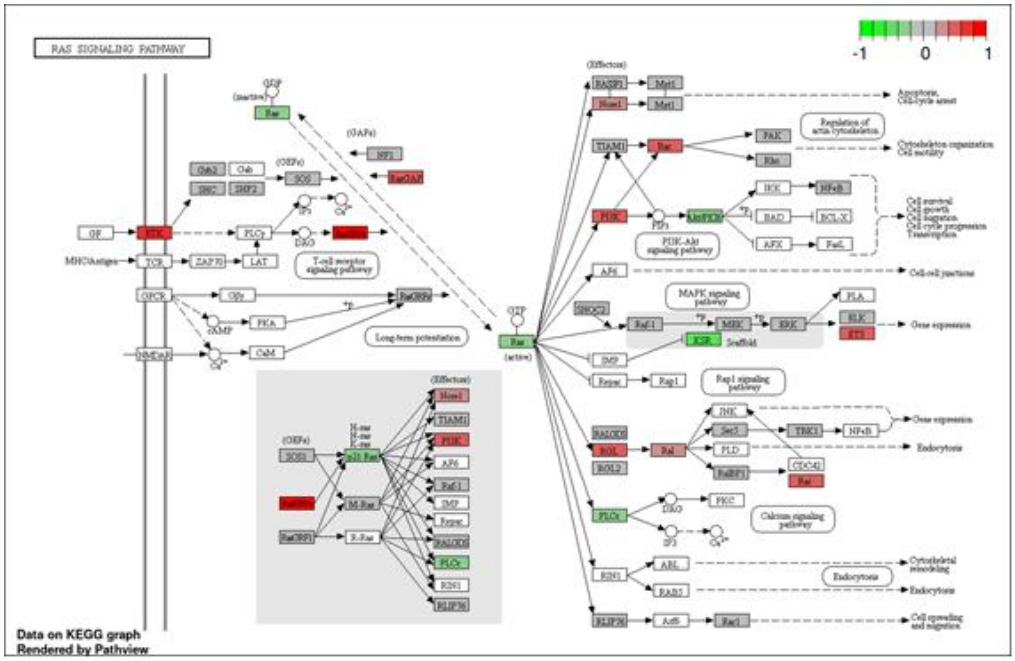

Fig.7: Upregulation of RAS-signalling pathway in KEGG

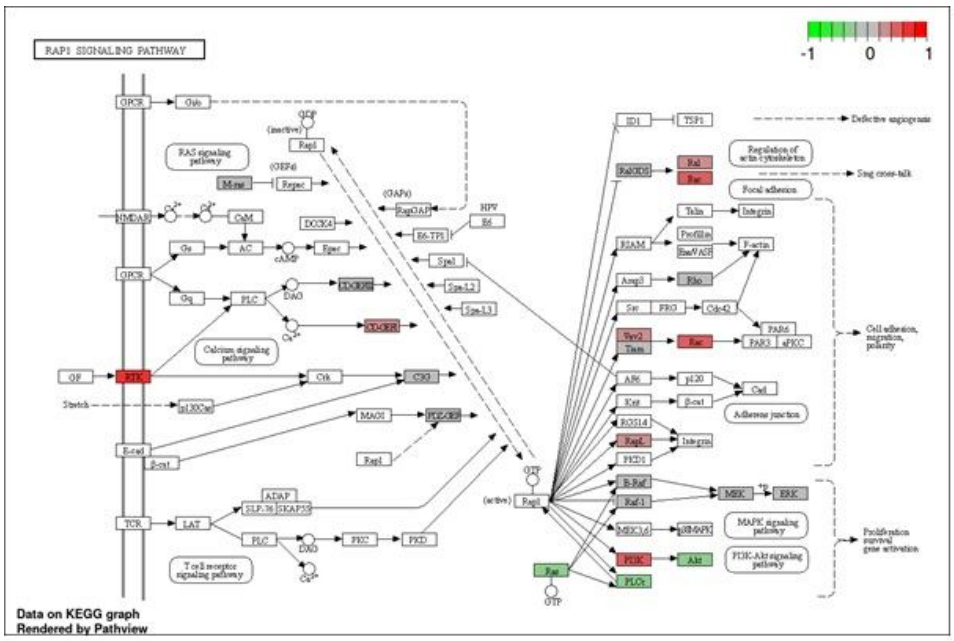

Fig.8: Up-regulation of RAP1 signalling pathway

DISCUSSION

The PTEN tumour suppressor gene, located on chromosome 10q23, is one of the most frequently mutated gene in cancer. Its germline mutations cause Cowden syndrome, which is characterized by an increased risk for the development of benign and malignant tumours. Sporadic PTEN mutations have been observed frequently in a variety of human neoplasm, including breast cancer.

Loss of function of the PTEN tumour suppressor can lead to constitutive activation of the PI3K/Akt/mTOR pathway. The PI3K/Akt/mTOR pathway has been shown to play important role in cancer by regulating apoptosis, neoangiogenesis and proliferation and has become an important target for anticancer drug development.

Development of therapeutic strategies for PTEN and RAS alteration is a challenge for many researchers. Pathways involving PTEN and RAS constitute a complex intertwining signalling network with multiple point of interaction and various feedback mechanism. Tumour with complete loss of PTEN and constitutive high-level activation of AKT may be refractory to inhibition of RAS signalling through the use of inhibitors, such as Iressa and Tarceva, which impinge on the RAS pathway upstream of PI3K.

Our metadata analysis evidenced that Ras-signalling pathway and RAP1 pathway makes tumour microenvironment with each other due to that resistance in cancer cell antitumour activity is found. Frequent activation of this pathway in cancer is failure response of drug which resist antitumour activity.

CONCLUSION

We conclude that when PTEN gene is mutated in Breast cancer many oncogenes and non-oncogenes are trigger and supressed. In our metadata study of TCGA Breast Invasive Carcinoma of Pan-cancer Atlas discover over-expression and suppression of oncogenic and non-oncogenic support system are interlinked which is playing crucial role in breast tumorigenesis. RAS-signalling pathway activates tumour microenvironments which is interlinked with many pathways for supporting breast cancer tumour progression, cell growth and proliferation. Furthermore, when taken in context with previously done studies failure of PTEN tumour suppressor gene in hyperactivation of many oncogenic pathways for promoting breast cancer. By inhibiting RAS pathway resistance in cancer therapies may be controlled.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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