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PTPN12 gene expression signature in triple negative breast cancer cohort

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Abstract

PTPN12 tyrosine phosphatase may play a role in tumor development/progression in triple negative breast cancer patients (TNP). The effects of PTPN12 appear to be mediated through several tyrosine kinase receptors including EGFR, HER2, and PDGFR-beta. We investigated the variability associated with PTPN12 transcript in the microarray gene expression data obtained from 105 TNP as determined by IHC for ER and PR and IHC and FISH for HER2 during our clinical molecular profiling on solid tumors. The mRNA levels of PTPN12 in our cohort was highly variable suggesting a complex genetic regulation of PTPN12 transcription in TNP patients. The highly variable nature of PTPN12 mRNA levels lead us to perform a correlation-based analysis of the transcriptome in TNP samples to gain insight into pathways and cellular processes associated with PTPN12 variation. Specifically, we quantile-normalized and performed two-dimensional hierarchical clustering of the 1000 top correlated genes with PTPN12 expression across the 105 TNP samples. We identified seven distinct gene clusters and three distinct patient subpopulations. The three distinct subtypes of TNP were comprised of low expressing PTPN12 (median log2 expression of 12.3), medium expressing PTPN12 (median log2 expression of 13.1), and high expressing PTPN12 (median log2 expression of 13.4). From the 7 gene clusters identified, 6 were positively correlated and one cluster (cluster 3) was negatively correlated with PTPN12 expression. Upon examining the genes within each cluster, we found that all contain unique set of genes related to cell proliferation, cell death, cell motility, cell cycle regulation and other cancer related pathways. From the 7 clusters, clusters 1, 6 and 7 had the highest fraction of such genes. The gene expression pattern and the gene content of these three clusters is as follows: Cluster 1 (208 genes) were genes that were highly expressed in all three TNP groups and the expression of the genes was highest in high PTPN12 expressing patients. Functional classification of genes by the DAVID bioinformatics tool at NCBI showed several genes related to cancer, including 19 MAP kinase signaling genes, and 15 genes involved in regulation of apoptosis. Cluster 6 (150 genes) contained genes showing very low expression in low-expressing TNPs, moderately expressed in medium expressing TNPs, and highly expressed in high-expressing TNPs. Cluster 6 had the highest fraction of cancer related genes including 7 mitosis and cell cycle check point genes, 6 cytoskeletal genes, 4 phosphatases and 6 kinases including KRAS, 4 DNA repair genes, 8 signal transduction genes, 3 positive regulator of apoptosis, and 22 genes involved in regulation of gene expression. Cluster 7 (166 genes) contained genes that were expressed at low level throughout the three TNP groups but the severity of under-expression was higher in TNP patients with low PTPN12 expression. In cluster 7 there were 5 genes belonging to the EGFR and FGF signaling pathways, and 6 genes involved in mitosis. It is worth noting that EGFR pathway has been postulated to crosstalk with PTPN12 protein. All together our results provide support for the involvement of PTPN12 in cancer development and highlights a promising therapeutic target for TNP patients.

Background

Triple Negative (TN) breast cancer is an aggressive subtype of breast cancer defined as the absence of Her2 gene amplification and/or expression, absence of ER expression, and absence of PR expression. In contrast to the other two major subtypes, the molecular mechanism underlying TN is poorly understood and no effective treatment for the TN patients currently exists. In the search for molecular targets, Sun et al (1) recently published identification of PTPN12 tyrosine phosphatase protein as a potential target for TN patients by performing a genetic screen for kinase and phosphatase molecules in Human Mammary Epithelial cells. This study suggested that PTPN12 acts as a tumor suppressor and its loss of function/expression promotes cell proliferation partly through hyperactivation of HER-signaling pathways.

In order to gain further insight into the molecular mechanism of PTPN12 and its relevance to TN breast cancer cases, we performed gene expression analysis of the 105 TN patients.

Methods

105 gene expression microarrays of triple negative breast cancer patients whose tissue samples were analyzed with Caris Target Now™ (Caris Life Sciences, Phoenix, AZ) molecular profiling test were selected for this study. The triple negative status of each patient was determined by IHC (for ER, PR, and Her2) and by FISH (for Her2). Gene expression was performed using illumina HT-12 platform which contains over twenty nine thousand probes representing ~18 thousand genes in the genome. Patient gene expression data were normalized using quantile normalization method. Following data normalization, we ranked genes based on the Spearman correlation p-values and selected top 1000 genes that were significantly correlated with PTPN12 mRNA expression levels. We then performed two-dimensional hierarchical clustering of the 1000 top correlated genes with PTPN12 expression across the 105 TNP samples. We also performed a comprehensive pathway analysis using all the genes as the input to the GSEA software (2,3). All the gene expression analyses were performed using R software (version 2.13.0).

Results

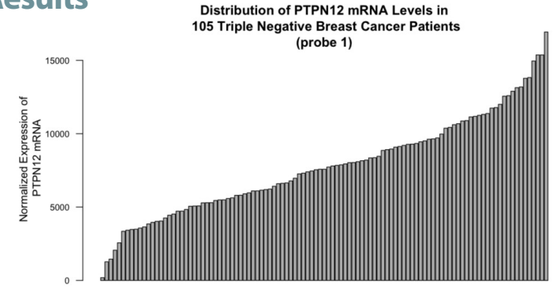


Figure 1 – Distribution of PTPN12 mRNA expression levels in 105 TN breast cancer patients

Distribution of PTPN12 mRNA levels as measured by Illumina HT-12 platform is shown in figure 1. This result demonstrated the presence of high variability in PTPN12 transcript levels among the TN patients. This high variability, which suggests complex genetic regulation, allowed us to carry correlation analysis at the transcriptome level followed by hierarchical clustering of the subset of transcripts that exhibited significant covariation with PTPN12 mRNA levels. For this analysis we selected top 1000 significantly correlated transcripts. The results of the two dimensional clustering is shown in Figure 2. This analysis identified three distinct subset of TN patients: Patients with a) low expression of PTPN12 (P1); intermediate expression of PTPN12 (P2); and high expression of PTPN12 (P3). Top 1000 significantly correlated transcripts divided into 7 clusters (denoted by C1-C7) each with a distinct pattern of expression. Functional classification of genes within each cluster revealed the presence of several oncogenes and tumor suppressor genes within each cluster. This observation supports the findings of the previous report in highlighting PTPN12 as a potential target for TN patients.

Closer examination of the top correlated genes revealed the presence of SOCS4, which has been recently associated with earlier tumor stage and better clinical outcome in breast cancer patients (4), and several proteosome genes including CBL among the PTPN12 top 1000 correlated genes. The positive association of PTPN12 with SOCS4 and CBL gene products in C2 cluster (shown in Figure 3) supports the hypothesis that PTPN12 may negate EGFR/Her2 signaling by promoting degradation of these receptors by ubiquitination. Consistent with this hypothesis, we found that when P1 and P3 patients are selected for pathway analysis, the proteosome pathway was the most differentially regulated (upregulated) pathway among over 800 pathways investigated by the GSEA pathway analysis software (Table 1 and Figure 4).

Figure 2 – Two-dimensional hierarchical clustering of the 1000 top correlated genes with PTPN12 mRNA expression across the 105 TNP samples. The three distinct patient clusters are denoted by P1, P2, and P3 and the seven distinct gene clusters are denoted by C1 through C7

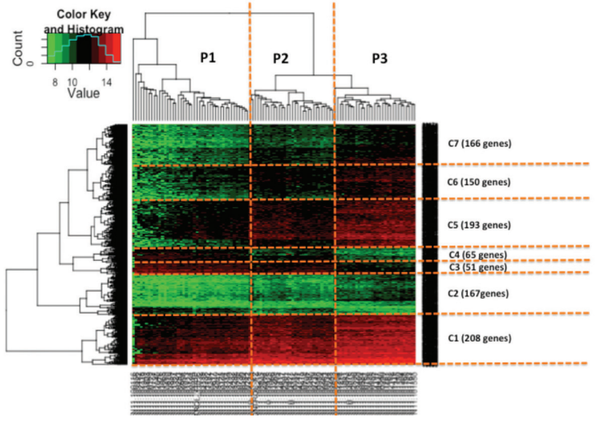


Figure 3 – PTPN12 is significantly correlated with SOCS4 (left panel) and CBL (right panel). Scatter plot colors represent the cluster membership of the sample (blue = P1 or low expressing PTPN12 cluster, grey = P2 or intermediate expressing PTPN12 cluster, red = P3 or high expressing PTPN12 cluster)

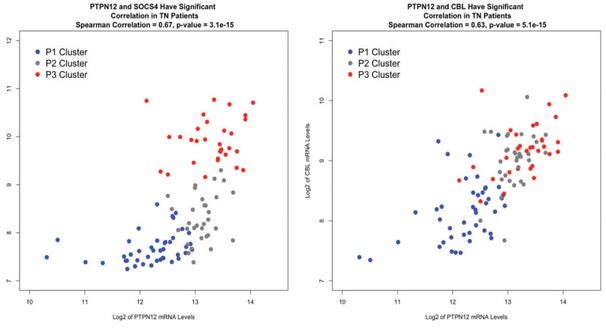
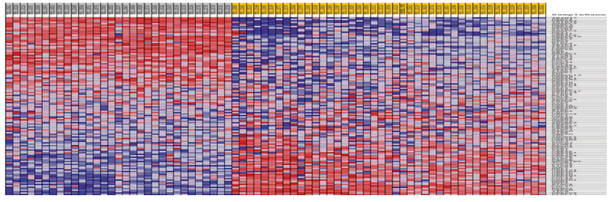


Table 1 – Top 25 Differentially Expressed Pathways between P1 and P3 clusters (GSEA Analysis)

Pathway	Source	# of Genes in Pathway	Number of Pathway Genes Differentially Expressed (% Total)	Nominal p-value
Proteasome Pathway	Biocarta	17	9 (53%)	<0.01
ATM Pathway	Biocarta	20	4 (20%)	0.02
Cyclin E Associated Events During G1 S Transition	Reactome	56	27 (48%)	<0.01
CDC20 Phospho Apc Mediated Degradation of Cyclin A	Reactome	61	22 (36%)	0.02
Vif Mediated Degradation of Apobec3G	Reactome	46	18 (39%)	0.02
Stabilization of p53	Reactome	45	18 (40%)	0.07
G1 S Transition	Reactome	97	40 (41%)	0.07
Regulation of Apc Activators Between G1 S and Early Anaphase	Reactome	68	23 (34%)	0.02
WNT Pathway	Biocarta	25	11 (44%)	<0.01
SCF SKP2 Mediated Degradation of P27 P21	Reactome	50	21 (42%)	<0.01
Signaling By WNT	Reactome	56	22 (39%)	0.02
Parkinsons Disease	KEGG	107	41 (38%)	0.02
P53 Independent DNA Damage Response	Reactome	42	17 (40%)	0.02
Regulation of Ornithine Decarboxylase	Reactome	46	17 (37%)	0.04
S Phase	Reactome	98	46 (47%)	<0.01
Autodegradation of CDH by CDH1 APC	Reactome	56	20 (36%)	0.05
Homologous Recombination Repair	Reactome	15	4 (27%)	<0.01
Proteasome	KEGG	42	16 (38%)	0.03
Ubiquitin Mediated Proteolysis	KEGG	124	33 (27%)	0.02
Oxidative Phosphorylation	KEGG	113	43 (38%)	0.02
M G1 Transition	Reactome	60	24 (40%)	0.08
Cell Cycle Checkpoints	Reactome	106	40 (38%)	0.04
SCF Beta TRCP Mediated Degradation of EMI1	Reactome	46	17 (37%)	0.02
Deadenylation of MRNA	Reactome	20	8 (40%)	0.04
Regulation of Beta Cell Development	Reactome	108	36 (33%)	0.02

Figure 4 – Expression pattern of 42 gene members of KEGG "Proteasome" pathway in TN patients with high (gray columns) and low (orange columns) PTPN12 expression.



Conclusions

1. PTPN12 is highly variable among the TN breast cancer patients.
2. Cell cycle genes significantly covary with PTPN12 mRNA and cell cycle pathways are among the most differentially expressed pathways between high and low PTPN12 expressing patients. This provides additional support that PTPN12 may be a bona fide target in subpopulation of TN patients.
3. Proteosome pathway is significantly activated in patients with high expression of PTPN12 supporting the hypothesis that the previously reported PTPN12 negation of EGFR/Her2 could be mediated partly by induction of proteasomal degradation of signaling receptors.

References

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