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Mapping of TP53 protein network using Cytoscape software

Asita Elengoe[1*], Salehhuddin Hamdan[2]

[1]Department of Biotechnology, Faculty of Science, Lincoln University College, 47301 Petaling Jaya, Selangor, Malaysia
[2]Department of Biosciences and Health Sciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia

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**Corresponding author:*

asitaelengoe@yahoo.com

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ABSTRACT

TP53 acts as a tumor suppressor in cancer. It induces cell cycle arrest or apoptosis in response to cellular stress and damage. p53 gene alteration could cause uncontrolled cell proliferation. In the present study, we used TP53 gene as the seed in the construction of a protein-protein interaction network to identify genes that might involve in tumorgenesis process with TP53. TP53 protein interaction database was obtained from STRING version 9.1 program. High-throughput experimental data, literature data and hypothetical studies have been used to determine the roles of candidate genes in TP53 pathway. A total 500 genes from STRING database loaded into Cytoscape version 2.8.3. The 1762 protein interactions were assembled and visualized in y organic form. We found eight specific non-overlapping clusters of various sizes, which emerged from the huge network of protein-interactors using MCODE version 1.32 clustering algorithm. Biological Networks Gene Ontology (BiNGO) was used to determine two ontologies (molecular function and biological process) involved in the protein network. Most of the genes mainly participated in gene and protein expression, cell signaling and metabolism. A better understanding of the relationship between the genes could aid in developing prognostic markers and better therapeutic strategies in cancer treatment.

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Introduction

According to World Cancer Factsheet (February 2017), cancer is one of the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases in 2012 (1). The number of new cases is expected to rise by about 70% over the next two decades. Moreover, lack of specific symptoms in the early stage of disease leading to delay in diagnosis. The success rate of conventional methods such as surgery, chemotherapy and radiotherapy to treat cancer has not been very high. Furthermore, these treatments could cause damage to normal cells, DNA which leads to mutation, mouth ulcerations, cognitive impairments, cardiomyopathy, liver failure, delayed nausea and kidney failure (2). Thus, there is an urgency in developing new approaches for the treatment of cancer patients. Several important pathways are thought to be involved in cancer such as ErbB signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, PARP signaling pathway and p53 pathway.

Cell cycle regulation is complex. Most of genes such as cyclins, cyclin dependent kinases, cyclin dependent inhibitors, protein kinases and tumor suppressor genes involved in cell cycle process and its regulation (3). The TP53 acts as a tumor suppressor in cancer. It is an important gene that induces cell cycle arrest or apoptosis in response to cellular stress and damage. p53 gene alteration could cause uncontrolled proliferation of cell. Moreover, p53 related genes such as BCL2, MDM2 and BAX have been involved in tumorgenesis process via p53 pathway (4-6).

TP53 plays a vital role in cell cycle control and apoptosis. Defective p53 could allow proliferation of abnormal cells which resulting in cancer. Most of all human tumors consist of p53 mutants.

In a normal cell, Akt, also known as protein kinase B (PKB), exists in an hypoactive state because of the low expression of HER2 or existence of functional phosphatase and tension homolog deleted on chromosome ten (PTEN) so the cell cycle progression remains through the negative G1 regulators such as nuclear p21/Cip1, WAF1, and p27Kip1. In addition, murine double minute gene 2 (MDM2) maintains in an inactive form. Therefore, it is unable to degrade p53 and activates the p21 Cip1/WAF1, which leading to G1 growth arrest and apoptosis. The p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: growth arrest, DNA repair and apoptosis (cell death). The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. During the growth arrest, p53 may activate the transcription of proteins involved in DNA repair. Apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA.

In a cancer cell, Akt exists in a hyperactive form due to high expression of HER2 or mutated PTEN. As a result, Akt phosphorylates p21Cip1/WAF1 at Threonine 145 within the nuclear localization signal (NLS) region (7-8). This causes cytoplasmic retention of p21Cip1/WAF1 and enhanced cell survival. p21Cip1/WAF1 is a potent inhibitor of CDK2 that contributes to G1 growth arrest and apoptosis. Similar to p21Cip1/WAF1 activity, p27Kip1 is phosphorylated by Akt at Threonine 157 within the NLS region and remains in the cytoplasm, contributing to suppression of apoptosis. In addition, Akt

also phosphorylates MDM2, a ubiquitin E3 ligase which induces its nuclear accumulation and p53 degradation ability, leading to down-regulated cell cycle progression and apoptosis. Researchers believed that many genes participated in the cell cycle regulation process are involved as promoter of tumorgenesis process. To understand the association of different genes and their protein products in the etiology of disease, the function played by interactions between p53 and genes that participated in different types of other pathways in the carcinogenesis process should be studied. Recently, molecular network are being used to predict novel possible genes depend on the hypothesis that genes that lie in the neighborhood of disease causing genes in the network are likely to be associated with same or similar disease (9). Based on Goehler (10) and Jonsson and Bates (11) studies, they have been demonstrated that novel disease genes were identified via the protein-protein interaction networks. Besides that, they indicated that genes involved in TP53 pathway are highly interconnected (12-13). Hence, one biomolecule alteration might affect the functioning of other associated biomolecules.

Moreover, Jayaraman and his co-workers identified that the TP53 gene was conserved across the mammalian genome using phylogenetic methods (14). TP53 gene alteration was probably because of the changes that accumulated during the evolutionary history of this gene. A better understanding of the TP53 biological networking will help in studying deregulations in TP53 pathway. Therefore, the objective of this study was to analyze the functional interactions of p53 gene and its interacting proteins.

Materials and Methods

Identification of genes involved in TP53 pathway

STRING version 9.1 program was used to find the genes participated in pathway of TP53 (15). This tool was selected because it has an extensive collection of pre-computed interaction data derived from different types of sources such as high-throughput experimental data, literature review data and predictions of computational; probalistic scoring was used to score the network interactions for getting higher confidence (>90%) in the interactions and; allows grouping of the interacting molecules into clusters using MCODE algorithms in the advanced mode. Neighbourhood, gene fusion, co-occurrence, co-expression, experiments, databases and text mining were chosen as the prediction methods in this analysis. The interactions which had a confidence score greater than 0.9, representing more than 80-90% confidence in the predictions was also filtered in this analysis (16).

Clustering of p53 gene analysis

MCODE version 1.32 clustering algorithm is one of the plugins in Cytoscape version 2.8.3 software. It was used for p53 gene clustering analysis. The MCODE version 1.32 clustering algorithm was applied to segregate the network into smaller subgroups of eight clusters. A cluster is a set of objects which share some common characteristics.

In protein-protein interaction networks, clusters correspond to two types of modules: protein complexes and functional modules. Protein complexes are groups of proteins that interact with each other at the same time and place, forming a single multi-molecular machine. Functional modules consist of proteins that participate in a particular cellular process while binding to each other at a different time and place.

Clustering in protein-protein interaction networks therefore involves identifying protein complexes and functional modules. This process has the following analytical benefits (17):

(1) clarification of PPI network structures and their component relationships;

(2) inference of the principal function of each cluster from the functions of its members;

(3) elucidation of possible functions of members in a cluster through comparison with the functions of other members.

The MCODE algorithm operates in three steps: vertex weighting, complex prediction, and optional postprocessing to filter or add proteins to the resulting complexes (17).

Determination of molecular function and biological processes of the genes involved in TP53 protein network

TP53 protein network was evaluated with BiNGO program which is embedded within the Cytoscape version 2.8.3 software. It was carried out to determine the molecular function and biological processes of the TP53 protein network.

Results

Identification of genes involved in TP53 pathway using STRING version 9.1 program

In this study, TP53 gene was used as the seed in the construction of a protein-protein functional association network to identify genes that might involve in tumorgenesis process with TP53. TP53 protein interaction database was obtained from STRING version 9.1 program. High-throughput experimental data, literature data and hypothetical studies have been used to determine the roles of candidate genes in TP53 pathway. In addition, STRING program also scores the network interactions using probabilistic scoring to get higher confidence in the interactions. In this study, hypothetical studies defined as prediction of protein-protein interaction based on text mining. Therefore, combination of databases from experimental and prediction sources provide a wider base for analyzing the protein interactome. A sum of 500 interacting

proteins with 1762 interactions was obtained from the database. Figure 1 shows the highly connected network of molecules. The protein-protein interaction was represented as network graph with proteins as nodes and interactions as edges. Most of genes were situated at the center of the network whereas few molecules loosely arranged at the periphery. Some of the interactors are connected to one another by multiple lines which represent interactions derived from more than one source of databases.

Gene clustering analysis using MCODE version 1.32 algorithm

Firstly, a total 500 genes from STRING database loaded into Cytoscape version 2.8.3 software. The 1762 protein interactions were assembled and visualized in y organic form. The MCODE version 1.32 clustering algorithm was applied to segregate the network into smaller subgroups of eight clusters (Figure 2). Table 1 shows clustering results of TP53 protein.

Evaluation of TP53 protein network using BiNGO analysis

After that, the TP53 protein network was analyzed with the BiNGO program which is one of the plugins in Cytoscape version 2.8.3 software. BiNGO was used to determine two ontologies (molecular function and biological process) involved in the protein network. Detail results were displayed in Table 2 and Figure S1.

Discussion

A highly connected network of 35 protein molecules was found in the first cluster. This cluster has more interactions with other proteins when compared with all other clusters. The proteins possess a wide range of functional attributes. Furthermore, TP53 is part of this cluster. This dense cluster consists of proteins of TP53 binding (CDKN2A, BLM, MDM2), cyclins (CDK1), cyclin dependent kinases (CDK1, CDK7), tumor suppressors (TP53), transcriptional activators (MAX, NOTCH1, AR, KAT2B, HDAC1, YY1, TP53, BRCA2, SMAD2, CDK7,

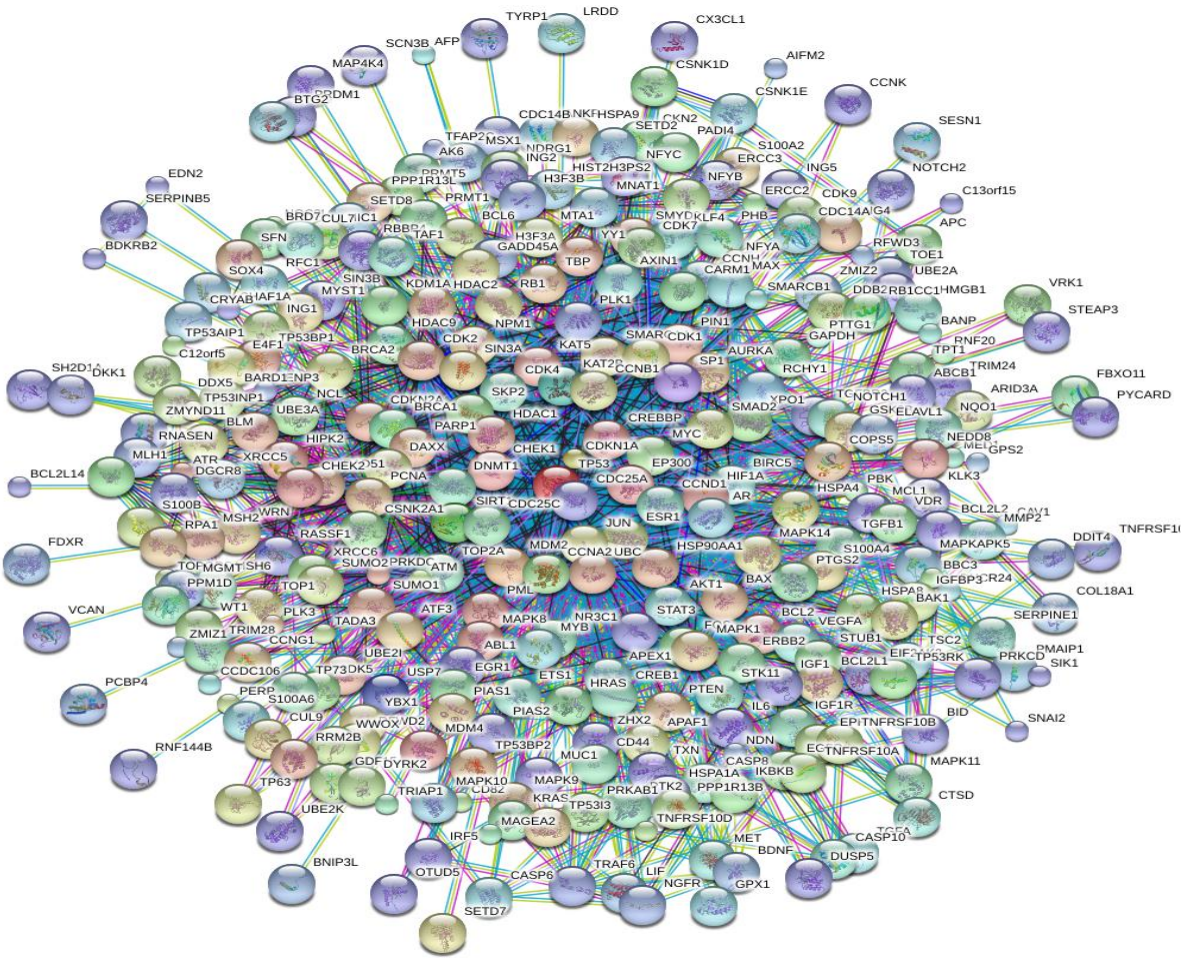


Figure 1 A total 500 genes from STRING database loaded into Cytoscape version 2.8.3. The 1762 protein interactions were assembled and visualized in y organic form.

STAT3, BRCA1, TGFB1), transcription factors (AR, KAT2B, CREB1, YY1, TP53, ESR1, SMAD2, CDK7, BRCA1, STAT3, MAPK1, MAX, HIF1A, CDKN2A, HDAC1), apoptosis regulators (TP53, MDM2), HIFIA regulators (HIF1A, TP53), cell cycle regulators (MAPK1, NOTCH1, AR, HIF1A, CD44, PLK1, MAPK14, ESR1, SMAD2, STAT3), DNA repair (NOTCH1, BLM, CREB1, TP53, BRCA2, MLH1, SMAD2), heat shock protein binding (CDK1, HIF1A) and many ubiquitin proteins.

Some important genes' functions were described as below. The cyclins and cyclin dependent kinase proteins are playing role in control of cell cycle progression. The alteration of these proteins affects progression and contributes to the uncontrolled cell proliferation which leads to cancer. CDKN2A protein is a key cell cycle protein. It is capable to enhance cell cycle arrest in G1 and G2 phases. It also acts as a tumor suppressor. It binds to MDM2 and blocks its nucleoplasmic shuttling by sequestering it in the nucleolus. This inhibits the oncogenic action of MDM2 by blocking MDM2- induced degradation of p53 and enhancing p53-dependent transactivation and apoptosis. It also induces G2 arrest and apoptosis in a p53-independent manner by preventing the activation of cyclin B1/CDC2 complexes. It binds to BCL6 and down-regulates BCL6-induced transcriptional repression.

BRCA1 plays an essential function in DNA damage repair. It also required for appropriate cell cycle arrests after ionizing irradiation in both the S-phase and the G2 phase of the cell cycle. Besides that, it also participated in p21 transcriptional regulation in response to DNA damage; to maintain the genomic stability. While, BRCA2 involved in double-strand break repair and homologous recombination. BAX induces apoptosis through binding to, and antagonizing the apoptosis repressor BCL2 or its adenovirus homolog E1B 19 kDa protein. Programmed cell death is accelerated by the cytochrome C release and CASP3 activation. PTEN is also a tumor suppressor protein. It acts as a dual-specificity protein phosphatase, dephosphorylating tyrosine-, serine- and threonine-phosphorylated proteins. Apart from that, it also acts a lipid phosphatase, removing the phosphate in the D3 position of the inositol ring from phosphatidylinositol 3,4,5-trisphosphate, phosphatidylinositol 3,4-diphosphate, phosphatidylinositol 3- phosphate and inositol 1,3,4,5-tetrakisphosphate with order of substrate preference in vitro PtdIns(3,4,5)P3 > PtdIns(3,4)P2 > PtdIns3P > Ins(1,3,4,5)P4. The lipid phosphatase activity is crucial for its tumor.

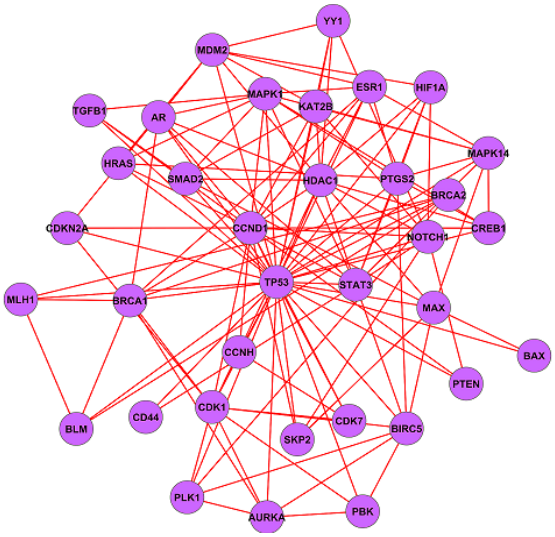
Notch1 plays role as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination. Notch intracellular domain (NCID) forms a transcriptional activator complex with RBP-J kappa through activation of ligand. Then, it activates genes of the enhancer of split locus. It hinders the differentiation implementation, proliferation and apoptotic process. The alteration of this gene contributes to transformation or progression in some T-cell neoplasms.

HIF1A acts a master transcriptional regulator of the adaptive response to hypoxia. Under hypoxic conditions, it activates the transcription genes such as erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor genes to facilitate metabolic adaptation to hypoxia. It plays crucial role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease. MDM2 is a negative regulator of TP53 protein which defines that it inhibits p53 and p73-mediated cell cycle arrest and apoptosis via binding its transcriptional activation domain. Therefore, it stabilized p53 protein through ubiquitination and permits p53 export for proteasome-mediated proteolysis. Moreover, p53 inactivation affects tumor suppression activity of p53. In differentiated cells, MAPK1 participated in both the initiation and regulation of meiosis, mitosis and post mitotic functions via phosphorylating a number of transcription factors including ELK1. It also plays role in translation initiation through phosphorylation of E1F4BP1.

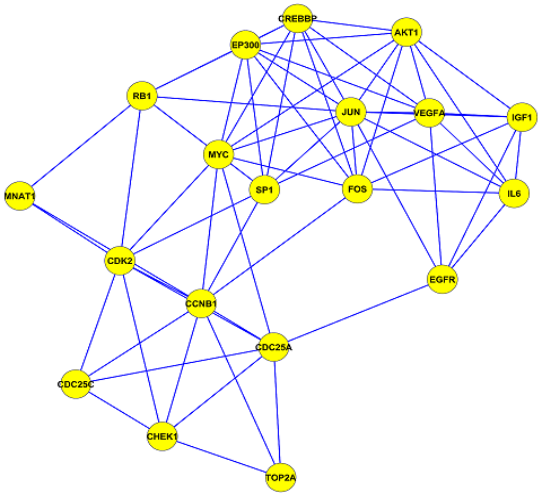
In the second cluster, 19 proteins play a variety of roles such as transcriptional activators, transcription factors, signal transducers, cyclin dependent kinases; and involved in cell growth, apoptosis, metabolism, differentiation and angiogenesis processes.

Several essential genes in this cluster were explained as follows. Akt1 is a general protein kinase that capable of phosphorylating several known proteins including TBC1D4. It signals downstream of phosphatidylinositol 3-kinase (PI(3)K) to mediate the effects of various growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin and insulin-like growth factor I (IGF-I). MYC plays an important role in regulates transcription of several genes that participated in a wide variety of functions including cell growth, metabolism, programmed cell-death, cell cycle, angiogenesis and differentiation. Alteration of this protein contributes not only its expression but also various target genes and their pathways. In spite of this, alteration of this protein has been reported in several cancers.

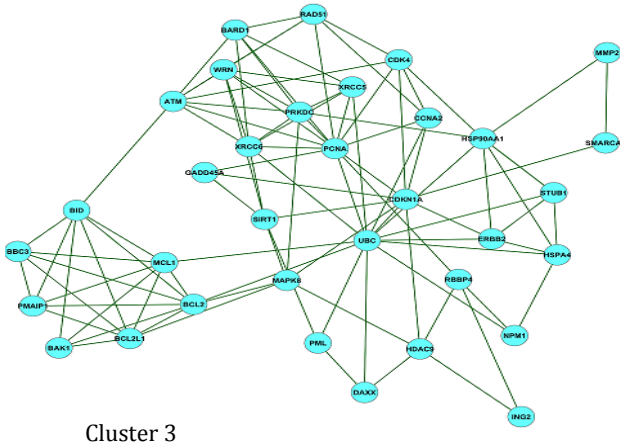
FOS is a nuclear phospho-protein which forms a tight but non-covalently linked complex with JUN/AP-1 transcription factor. It acts as a regulator for the development of cells destined to form and maintain the skeleton. In addition, it also has a crucial role in transduction of signal, cell proliferation and differentiation. VEGFA protein is a growth factor which participated actively in angiogenesis, vasculogenesis through mediation of vascular permeability and endothelial cell growth. Angiogenesis is a physiological process via which new blood vessels form from the old vessels. It also induces cell migration and inhibits apoptosis. This protein plays a critical function in



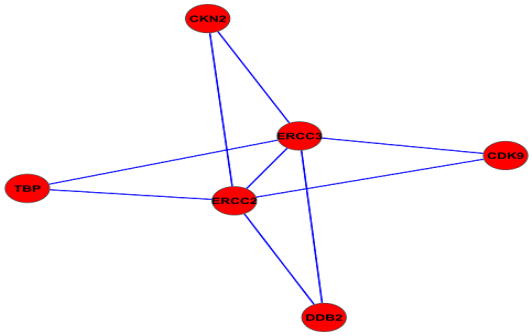
Cluster 1



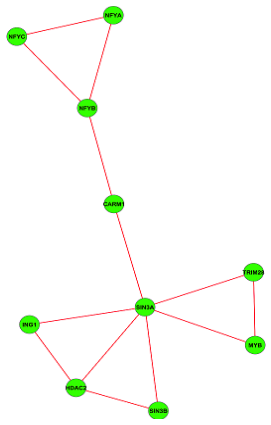
Cluster 2



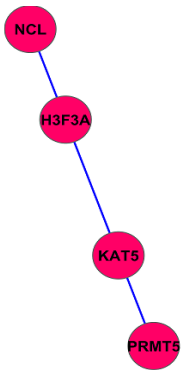
Cluster 3



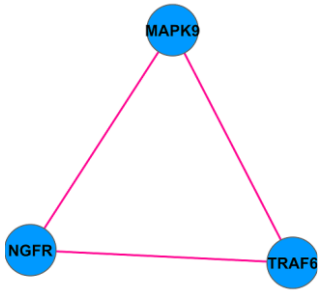
Cluster 4



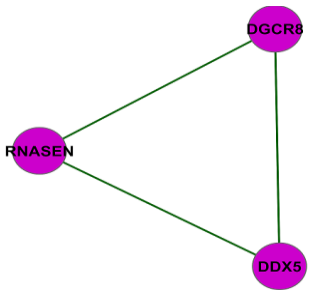
Cluster 5



Cluster 6



Cluster 7



Cluster 8

Figure 2 Eight specific non-overlapping clusters of various sizes, which emerged from the huge network of protein-interactors, were determined using MCODE version 1.32 clustering algorithm.

Table 1. Genes, score, nodes and edges for each cluster

Cluster	Genes	Score	Nodes (Proteins)	Edges (Interaction)
1	CD44,HDAC1, PLK1,CDK1, TP53, BLM, YY1, SKP2, BRCA1, CCND1, MAX, NOTCH1, PBK, HRAS, CDNK2A, MAPK1, MAPK14, ESR1, PTEN, AURKA, AR, MDM2, MLH1, IREB1, BAX, BIRC5, PTGS2, HIF1A, SMAD2, KAT2B, STAT3, CCNH,CDK7, TGFB1, BRCA2	3.771	35	132
2	CREBBP, AKT1, EP300, RB1, MNAT1, CDK2, CDC25C, CHEK1, CCNB1, CDC25A, MYC, SP1, JUN, FOS, VEGFA, IGF1, IL6, EGFR, TOP2A	3.474	19	66
3	CDKN1A, BAK1, BCL2L1, WRN, PMA1P1, XRCC5, HSP90AA1, XRCC6, BCL2, MCL1, BBC3, RAD51, STUB1, RBBP4, CCNA2, ING2, ERRB2, BARD1, MAPK8, PLNA, PMC, MMP2, SMARCA4, NPM1, UBC, CDK4, ATM, HDAC9, PRKDC, HSPA4, SIRT1, DAXX, BID, GADD45A	2.941	34	100
4	CKN2, ERCC3, CDK9, ERCC2, TBP, DDB2	1.500	6	9
5	NFYC, NFYB, NFYA, CARM1, SIN3A, TRIM38, MYB, SIN3B, HDAC2, ING1	1.300	10	13
6	NCL, H3F3A, KAT5, PRMT5	1.000	4	4
7	MAPK9, NGFR, TRAF6	1.000	3	3
8	DGCR8, RNASEN, DDX5	1.000	3	3

Table 2 Molecular function of genes involved in (A) Cluster 1; (B) Cluster 2; (C) Cluster 3; (D) Cluster 4; (E) Cluster 5; (F) Cluster 6; (G) Cluster 7; and (H) Cluster 8

(A) Cluster 1

Molecular function	Genes
Enzyme binding	KAT2B TP53 BRCA2 AURKA BIRC5 SMAD2 PTEN BRCA1 STAT3 MAPK1 CCND1 HIF1A CDKN2A HDAC1 PLK1 MDM2
Transcription factor binding	AR KAT2B CREB1 YY1 TP53 ESR1 SMAD2 CDK7 BRCA1 STAT3 MAPK1 MAX HIF1A CDKN2A HDAC1
Transcription activator activity	MAX NOTCH1 AR KAT2B HDAC1 YY1 TP53 BRCA2 SMAD2 CDK7 STAT3 BRCA1 TGFB1
Protein binding	HRAS BLM PTGS2 MLH1 AURKA PTEN TGFB1 MAX CDKN2A CD44 CDK1 AR KAT2B CCNH CREB1 YY1 TP53 SKP2 ESR1 BRCA2 BIRC5 SMAD2 CDK7 PBK STAT3 BRCA1 MAPK1 CCND1 NOTCH1 HIF1A HDAC1 PLK1 MAPK14 BAX MDM2
Protein kinase binding	MAPK1 CCND1 KAT2B CDKN2A PLK1 TP53 AURKA STAT3
Transcription regulator activity	AR KAT2B CREB1 YY1 TP53 ESR1 BRCA2 SMAD2 CDK7 STAT3 TGFB1 BRCA1 MAX NOTCH1 HIF1A HDAC1 MDM2
Kinase binding	MAPK1 CCND1 KAT2B CDKN2A PLK1 TP53 AURKA STAT3
Structure-specific DNA binding	NOTCH1 BLM CREB1 TP53 BRCA2 MLH1 SMAD2
Promoter binding	MAX AR YY1 ESR1 TP53 SMAD2
DNA regulatory region binding	MAX AR YY1 ESR1 TP53 SMAD2
Sequence-specific DNA binding	MAX NOTCH1 AR HIF1A YY1 CREB1 ESR1 TP53 SMAD2 STAT3
Receptor signalling protein activity	MAPK1 CD44 PLK1 MAPK14 SMAD2 STAT3
Double-stranded DNA binding	BLM CREB1 TP53 MLH1 SMAD2
DNA binding	AR BLM CREB1 YY1 TP53 ESR1 MLH1 BRCA2 SMAD2 STAT3 BRCA1 MAPK1 MAX NOTCH1 HIF1A CDKN2A HDAC1
Protein dimerisation activity	MAX AR HIF1A BAX CREB1 TP53 BIRC5 STAT3 TGFB1
Transcription factor activity	MAX NOTCH1 AR HIF1A HDAC1 YY1 CREB1 ESR1 TP53 SMAD2 STAT3
Protein heterodimerisation activity	MAX HIF1A BAX TP53 BIRC5 TGFB1
Cyclin-dependent protein kinase regulator activity	CCND1 KAT2B CDKN2A
Ubiquitin protein ligase binding	TP53 SMAD2 AURKA BRCA1
p53 binding	CDKN2A BLM MDM2
DNA strand annealing activity	BLM TP53

RNA polymerase II carboxy-terminal domain kinase activity	CDK1 CDK7
Protein kinase activity	MAPK1 CDK1 CCND1 PLK1 MAPK14 AURKA PBK CDK7
Protein serine/threonine kinase activity	MAPK1 CDK1 PLK1 MAPK14 AURKA PBK CDK7
Phosphotransferase activity, alcohol group as acceptor	MAPK1 CDK1 CCND1 PLK1 MAPK14 AURKA PBK CDK7
Transcription coactivator activity	MAX KAT2B YY1 CDK7 BRCA1
Transcription cofactor activity	MAX KAT2B YY1 CREB1 CDK7 BRCA1
Protein complex binding	MAX CCND1 PLK1 ESR1 CDK7
Kinase activity	MAPK1 CDK1 CCND1 PLK1 MAPK14 AURKA PBK CDK7
Single-stranded DNA binding	BLM TP53 BRCA2
Nucleic acid binding	AR BLM CREB1 YY1 TP53 ESR1 MLH1 BRCA2 SMAD2 STAT3 BRCA1 MAPK1 MAX NOTCH1 HIF1A CDKN2A HDAC1
Histone acetyltransferase binding	HIF1A TP53
Cyclin-dependent protein kinase inhibitor activity	KAT2B CDKN2A
Receptor signalling protein serine/threonine kinase activity	MAPK1 PLK1 MAPK14
Binding	HRAS BLM PTGS2 MLH1 AURKA PTEN TGFB1 MAX CDKN2A CD44 CDK1 AR KAT2B CCNH CREB1 YY1 TP53 SKP2 ESR1 BRCA2 BIRC5 SMAD2 CDK7 PBK STAT3 BRCA1 MAPK1 CCND1 NOTCH1 HIF1A HDAC1 PLK1 MAPK14 BAX MDM2
MAP kinase activity	MAPK1 MAPK14
Transferase activity, transferring phosphorus-containing groups	MAPK1 CDK1 CCND1 PLK1 MAPK14 AURKA PBK CDK7
Transforming growth factor beta receptor binding	SMAD2 TGFB1
Protein serine/threonine kinase inhibitor activity	KAT2B CDKN2A
Protein kinase regulator activity	CCND1 KAT2B CDKN2A
ATP binding	MAPK1 CDK1 BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Adenyl ribonucleotide binding	MAPK1 CDK1 BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Kinase regulator activity	CCND1 KAT2B CDKN2A
Tubulin binding	BRCA2 BIRC5 BRCA1
Protease binding	TP53 BRCA2
Purine ribonucleotide binding	MAPK1 CDK1 HRAS BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Ribonucleotide binding	MAPK1 CDK1 HRAS BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Cyclin-dependent protein kinase activity	CDK1 CDK7
Adenyl nucleotide binding	MAPK1 CDK1 BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Androgen receptor binding	CDK7 BRCA1
Chaperone binding	TP53 BIRC5
Lipid binding	AR PTGS2 BAX ESR1 PTEN
POU domain binding	AR
Estrogen response element binding	ESR1
MAP kinase 2 activity	MAPK1
Inositol-1,3,4,5-tetrakisphosphate 3-phosphatase activity	PTEN
Phosphatidylinositol-3,4-biphospahte 3-phosphatase activity	PTEN
Ubiquitin-protein ligase inhibitor activity	CDKN2A
Ligase inhibitor activity	CDKN2A
Phosphoprotein binding	MAPK1 CD44
Purine nucleoside binding	MAPK1 CDK1 BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Nucleoside binding	MAPK1 CDK1 BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Purine nucleotide binding	MAPK1 CDK1 HRAS BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Protein kinase inhibitor activity	KAT2B CDKN2A
Enhancer binding	HIF1A CREB1
RNA polymerase II transcription factor activity, enhancer binding	HIF1A CREB1

Specific transcriptional repressor activity	HDAC1 YY1
Kinase inhibitor activity	KAT2B CDKN2A
Histone deacetylase binding	KAT2B HDAC1
Identical protein binding	HDAC1 BAX MDM2 BIRC5 BRCA1 TGFB1
Androgen receptor activity	AR
Anaphase-promoting complex binding	PLK1
Phosphatidylinositol-3,4,5 triphosphatase activity	PTEN
Phosphatidylinositol-3-phosphatase activity	PTEN
Four-way junction helicase activity	BLM
Guanine/thymine mispair binding	MLH1
Prostaglandin-endoperoxide synthase activity	PTGS2
Histone deacetylase regulator activity	TP53
Basal transcription repressor activity	MDM2
Estrogen receptor activity	ESR1
BH3 domain binding	BAX
Steroid hormone receptor binding	CDK7 BRCA1
DNA-dependent ATPase activity	BLM CDK7
Steroid hormone receptor activity	AR ESR1
Ligand-dependent nuclear receptor activity	AR ESR1
Hormone binding	AR ESR1
Polo kinase activity	PLK1
MP kinase activity	MAPK14
Aldehyde reductase activity	AR
Cobalt ion binding	BIRC5
MDM2 binding	CDKN2A
G-quadruplex DNA binding	BLM
Chromatin binding	NOTCH1 TP53 SMAD2
Nucleotide binding	MAPK1 CDK1 HRAS BLM PLK1 MAPK14 TP53 MLH1 AURKA PBK CDK7
Androgen binding	AR
Phosphatidylinositol triphosphate phosphatase activity	PTEN
ATP-dependent 3'-5' DNA helicase activity	BLM
Ubiqutin-protein ligase regulator activity	CDKN2A
Ligase regulator activity	CDKN2A
Transferase activity	MAPK1 CDK1 CCND1 KAT2B PLK1 MAPK14 AURKA PBK CDK7
Steroid binding	AR ESR1
Transforming growth factor beta receptor, pathway-specific cytoplasmic mediator activity	SMAD2
Hematopoietin/interferon-class (D200-domain) cytokine receptor signal transducer	STAT3
3'-5' DNA helicase activity	BLM
Bubble DNA binding	BLM
Nitric oxide synthase regulator activity	ESR1
Mitogen-activated protein kinase binding	MAPK1
BH domain binding	BAX
Enzyme regulator activity	CCND1 KAT2B CDKN2A ESR1 TP53 BIRC5
Heat shock protein binding	CDK1 HIF1A
Protein N-terminus binding	TP53 TGFB1
Death domain binding	BAX

SAP kinase activity	MAPK14
Hsp70 protein binding	CDK1
Nuclear hormone receptor binding	CDK7 BRCA1
Type II transforming growth factor beta receptor binding	TGFB1
Type I transforming growth factor beta receptor binding	SMAD2
Histone kinase activity	CDK1
Receptor binding	AR SMAD2 CDK7 PTEN BRCA1 TGFB1
Signal transducer activity	MAPK1 NOTCH1 AR HIF1A CD44 PLK1 MAPK14 ESR1 SMAD2 STAT3
Molecular transducer activity	MAPK1 NOTCH1 AR HIF1A CD44 PLK1 MAPK14 ESR1 SMAD2 STAT3
Hormone receptor binding	CDK7 BRCA1
co-SMAD binding	SMAD2
Chromatin DNA binding	NOTCH1
DNA secondary structure binding	BLM
Lipid phosphatase activity	PTEN
Phosphatidylinositol biphosphate phosphatase activity	PTEN
Hsp90 protein binding	HIF1A
Cyclin binding	CDK1
I-SMAD binding	SMAD2
Phosphotyrosine binding	MAPK1
Transforming growth factor beta receptor, cytoplasmic mediator activity	SMAD2
Protein phosphatase 2A binding	TP53
R-SMAD binding	SMAD2
Transmembrane receptor protein serine/threonine kinase signalling protein activity	SMAD2
Ran GTPase binding	BIRC5
Gamma-tubulin binding	BRCA2
Enzyme inhibitor activity	KAT2B CDKN2A BIRC5
Platelet-derived growth factor receptor binding	PTEN
Caspase inhibitor activity	BIRC5

(B) Cluster 2

Molecular function	**Genes**
Promoter binding	FOS EP300 SP1 JUN MYC
DNA regulatory region binding	FOS EP300 SP1 JUN MYC
SMAD binding	FOS EP300 JUN CREBBP
Double-stranded DNA binding	EGFR FOS SP1 JUN
Protein binding	EGFR IL6 CREBBP IGF1 CHEK1 RB1 CDC25C CDK2 CDC25A CCNB1 AKT1 FOS MNAT1 EP300 SP1 JUN VEGFA MYC TOP2A
Protein heterodimerisation activity	EGFR FOS JUN VEGFA TOP2A
Enzyme binding	AKT1 CCNB1 EGFR EP300 SP1 RB1 TOP2A
Peroxisome proliferator activated receptor binding	EP300 CREBBP
Transcription activator activity	EP300 SP1 JUN CREBBP RB1 MYC
Structure-specific DNA binding	EGFR FOS SP1 JUN
bHLH transcription factor binding	EP300 CREBBP
Protein dimerisation activity	EGFR FOS SP1 JUN VEGFA TOP2A
R-SMAD binding	FOS JUN
Kinase binding	CCNB1 EP300 RB1 TOP2A
Transcription coactivator activity	EP300 JUN CREBBP RB1

Identical protein binding	AKT1 EGFR SP1 VEGFA TOP2A CDK2
Histone acetyltransferease activity	EP300 CREBBP
Lysine N-acetyltransferase activity	EP300 CREBBP
Protein kinase activity	AKT1 CCNB1 EGFR CHEK1 CDK2
Receptor binding	IL6 EP300 VEGFA CREBBP IGF1 RB1
Transcription factor activity	FOS SP1 JUN CREBBP RB1 MYC
Sequence-specific DNA binding	FOS EP300 SP1 JUN MYC
DNA binding	EGFR FOS EP300 SP1 JUN CREBBP RB1 MYC TOP2A
Transcription cofactor activity	EP300 JUN CREBBP RB1
Growth factor activity	IL6 VEGFA IGF1
Phosphotransferase activity, alcohol group as acceptor	AKT1 CCNB1 EGFR CHEK1 CDK2
Histone deacetylase binding	SP1 TOP2A
Protein kinase binding	CCNB1 EP300 TOP2A
MRF binding	CREBBP
MyoD binding	CREBBP
Steroid hormone receptor binding	EP300 RB1
Transcription regulator activity	FOS EP300 SP1 JUN CREBBP RB1 MYC
Protein serine/threonine kinase activity	AKT1 EGFR CHEK1 CDK2
Kinase activity	AKT1 CCNB1 EGFR CHEK1 CDK2
N-acetyltransferase activity	EP300 CREBBP
Transferase activity	AKT1 CCNB1 EGFR EP300 CREBBP CHEK1 CDK2
MAP/ERK kinase activity	EGFR
Vascular endothelial growth factor receptor 1 binding	VEGFA
Epidermal growth factor receptor activity	EGFR
Acetyltransferase activity	EP300 CREBBP
Protein complex binding	CCNB1 EP300 IGF1
RNA polymerase II transcription factor activity	FOS SP1 JUN
N-acetyltransferase activity	EP300 CREBBP
Transcription factor binding	EP300 JUN CREBBP RB1
Transferase activity, transferring phosphorus-containing groups	AKT1 CCNB1 EGFR CHEK1 CDK2
Vascular endothelial growth factor receptor 2 binding	VEGFA
Nuclear hormone receptor binding	EP300 RB1
Growth factor receptor binding	IL6 VEGFA
Interleukin-6 receptor binding	IL6
DNA topisomerase (ATP-hydrolysing) activity	TOP2A
Hormone receptor binding	EP300 RB1
Nitric-oxide synthase regulator activity	AKT1
E-box binding	MYC
Protein tyrosine phosphatase activity	CDC25C CDC25A
Glucocorticoid receptor binding	EP300
Vascular endothelial growth factor receptor binding	VEGFA
DNA topoisomerase activity	TOP2A
Mitogen-activated protein kinase binding	EP300
Histone kinase activity	CCNB1

Nucleic acid binding	EGFR FOS EP300 SP1 JUN CREBBP RB1 MYC TOP2A
Protein homodimerisation activity	SP1 VEGFA TOP2A
Histone acetyltransferase binding	SP1
Fibronectin binding	VEGFA
Phosphatidylinositol-3,4-biphosphate binding	AKT1
Protein C-terminus binding	SP1 TOP2A
Binding	EGFR IL6 CREBBP IGF1 CHEK1 RB1 CDC25C CDK2 CDC25A CCNB1 AKT1 FOS MNAT1 EP300 SP1 JUN VEGFA MYC TOP2A
Platlet-derived growth factor receptor binding	VEGFA
Insulin-like growth factor binding	IGF1
Phosphoprotein phosphatase activity	CDC25C CDC25A
Chemo attractant activity	VEGFA
Receptor signalling protein tyrosine kinase activity	EGFR
Chromatin binding	CREBBP TOP2A
Phosphatidylinositol-3,4,5-triphosphate binding	AKT1
Acyltransferase activity	EP300 CREBBP
Transferase activity. Transferring acyl groups other than amino-acyl groups	EP300 CREBBP
Cytokine receptor binding	IL6 VEGFA
NF-kappaB binding	EP300
Trnasferase activity, transferring acyl groups	EP300 CREBBP
MAP kinase activity	EGFR
WW domain binding	CDC25C
Cytokine activity	IL6 VEGFA

(C) Cluster 3

Molecular function	**Genes**
Identical protein binding	HSP90AA1 ERBB2 PML BCL2L1 WRN STUB1 DAXX SIRT1 ATM RAD51 BAK1 BCL2 NPM1 SMARCA4 BARD1
Protein dimerisation activity	BAK1 HSP90AA1 MCL1 BCL2 ERBB2 NPM1 PML WRN BCL2L1 DAXX STUB1 ATM BARD1
Protein heterodimerisation activity	BAK1 MCL1 BCL2 ERBB2 NPM1 PML BCL2L1 BARD1
Protein binding	BID XRCC5 ING2 MCL1 ERBB2 XRCC6 PML PRKDC PMAIP1 BCL2L1 MMP2 STUB1 DAXX BAK1 BCL2 NPM1 CCNA2 RBBP4 HSP90AA1 WRN CDK4 SIRT1 ATM RAD51 CDKN1A BBC3 PCNA MAPK8 HDAC9 GADD45A SMARCA4 BARD1
DNA-dependent ATPase activity	XRCC5 XRCC6 WRN SMARCA4 RAD51
Protein homodimerisation activity	HSP90AA1 BCL2 NPM1 PML WRN DAXX STUB1 BARD1
ATP binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 ATM RAD51 CDKN1A HSPA4 MAPK8 SMARCA4
Enzyme binding	RBBP4 BCL2 ERBB2 PML HDAC9 SIRT1 DAXX STUB1 BARD1
Histone binding	RBBP4 NPM1 SIRT1 SMARCA4
Adenyl ribonucleotide binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 ATM RAD51 CDKN1A HSPA4 MAPK8 SMARCA4
Adenyl nucleotide binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 ATM RAD51 CDKN1A HSPA4 MAPK8 SMARCA4
Structure-specific DNA binding	XRCC5 XRCC6 PCNA WRN RAD51
Purine nucleoside binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 ATM RAD51 CDKN1A HSPA4 MAPK8 SMARCA4
DNA-dependent protein kinase activity	PRKDC ATM
TPR domain binding	HSP90AA1 STUB1
Nucleoside binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 ATM RAD51 CDKN1A HSPA4 MAPK8 SMARCA4
Protein C-terminus binding	XRCC5 ERBB2 XRCC6 SIRT1 RAD51
p53 binding	SIRT1 DAXX SMARCA4
ATP-dependent DNA helicase activity	XRCC5 XRCC6 WRN
BH domain binding	BCL2 BCL2L1

Double-stranded DNA binding	XRCC5 XRCC6 PCNA RAD51
Purine ribonucleotide binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 ATM RAD51 CDKN1A HSPA4 MAPK8 SMARCA4
Ribonucleotide binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 ATM RAD51 CDKN1A HSPA4 MAPK8 SMARCA4
DNA helicase activity	XRCC5 XRCC6 WRN
Purine nucleotide binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 ATM RAD51 CDKN1A HSPA4 MAPK8 SMARCA4
Transcription factor binding	BCL2 NPM1 PML PRKDC HDAC9 SIRT1 DAXX
Nucleotide binding	XRCC5 HSP90AA1 ERBB2 XRCC6 PRKDC WRN CDK4 SIRT1 ATM RAD51 CDKN1A MAPK8 HSPA4 SMARCA4
Protein complex binding	CDKN1A ING2 PCNA WRN ATM
Protein domain specific binding	CDKN1A HSP90AA1 BCL2 BCL2L1 SIRT1 STUB1
Hsp90 protein binding	ERBB2 STUB1
ATPase activity, coupled	XRCC5 XRCC6 WRN SMARCA4 RAD51
Helicase activity	XRCC5 XRCC6 WRN SMARCA4
Transcription activator activity	ING2 BCL2 NPM1 XRCC6 PML SMARCA4
Ubiqutin protein ligase binding	ERBB2 PML DAXX
ATPase activity	XRCC5 XRCC6 WRN SMARCA4 RAD51
Histone deacetylase activity	HDAC9 SIRT1
Protein deacetylase activity	HDAC9 SIRT1
Heat shock protein binding	ERBB2 DAXX STUB1
Protein N-terminus binding	DAXX ATM SMARCA4
Binding	BID XRCC5 ING2 MCL1 ERBB2 XRCC6 PML PRKDC PMAIP1 BCL2L1 MMP2 STUB1 DAXX BAK1 BCL2 NPM1 HSPA4 CCNA2 RBBP4 HSP90AA1 WRN CDK4 SIRT1 ATM RAD51 CDKN1A BBC3 PCNA MAPK8 HDAC9 GADD45A SMARCA4 BARD1
Deacetylase activity	HDAC9 SIRT1
Protein kinase activity	CDKN1A ERBB2 PRKDC MAPK8 CDK4 ATM
ATP-dependent helicase activity	XRCC5 XRCC6 WRN
Purine NTP-dependent helicase activity	XRCC5 XRCC6 WRN
Protein serine/threonine kinase activity	CDKN1A PRKDC MAPK8 CDK4 ATM
Protein channel activity	MCL1
5'-deoxyribose-5-phosphate lyase activity	XRCC6
Purine-specific mismatch base pair DNA N-glycosylase activity	PCNA
Dinucleotide insertion or deletion binding	PCNA
DNA polymerase processivity factor activity	PCNA
DNA binding	XRCC5 ING2 BCL2 ERBB2 XRCC6 PCNA PML PRKDC WRN ATM SMARCA4 RAD51
Phosphotransferase activity, alcohol group as acceptor	CDKN1A ERBB2 PRKDC MAPK8 CDK4 ATM
Histone deacetylase binding	RBBP4 HDAC9
Transcription regulator activity	ING2 BCL2 NPM1 XRCC6 PML HDAC9 SIRT1 DAXX SMARCA4
Epidermal growth factor receptor activity	ERBB2
Mismatch base pair DNA N-glycosylase activity	PCNA
DNA insertion or deletion binding	PCNA
Four-way junction helicase activity	WRN
Tat protein binding	NPM1
BH3 domain binding	BCL2
Kinase activity	CDKN1A ERBB2 PRKDC MAPK8 CDK4 ATM
Receptor signalling protein activity	ERBB2 MAPK8 DAXX
SMAD binding	PML STUB1
Nucleic acid binding	XRCC5 ING2 ERBB2 XRCC6 PML PRKDC WRN ATM RAD51 BCL2 NPM1 PCNA SMARCA4 BARD1
Transcription repressor activity	PML HDAC9 SIRT1 DAXX

NAD-dependent histone deacetylase activity	SIRT1
NAD-dependent protein deacetylase activity	SIRT1
Cobalt ion binding	PML
Single-stranded DNA-dependent ATPase activity	RAD51
JUN kinase activity	MAPK8
MutLalpha complex binding	PCNA
Y-form DNA binding	WRN
G-quadruplex DNA binding	WRN
Ribosomal large subunit binding	NPM1
Hydrolase activity, acting on carbon-nitrogen (but not peptides)	HDAC9 SIRT1
Transcription cofactor activity	NPM1 PML HDAC9 SIRT1
ATP-dependent 3'-5' DNA helicase activity	WRN
Mismatch repair complex binding	PCNA
ErbB-3 class receptor binding	ERBB2
Ribosomal small subunit binding	NPM1
Kinase binding	HDAC9 STUB1 BARD1
Transferase activity, transferring phosphorus-containing	CDKN1A ERBB2 PRKDC MAPK8 CDK4 ATM
Histone acetyl-lysine binding	SMARCA4
3'-5' DNA helicase activity	WRN
Misfolded protein binding	STUB1
Bubble DNA binding	WRN
Nitric-oxide synthase regulator activity	HSP90AA1
Death domain binding	BCL2
SAP kinase activity	MAPK8
Hsp70 protein binding	STUB1
Ubiquitin-ubiquitin ligase activity	STUB1
Nucleoside-triphosphatase activity	XRCC5 XRCC6 WRN SMARCA4 RAD51
DNA secondary structure binding	WRN
Pyrophosphatase activity	XRCC5 XRCC6 WRN SMARCA4 RAD51
Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	XRCC5 XRCC6 WRN SMARCA4 RAD51
Hydrolase activity, acting on acid anhydrides	XRCC5 XRCC6 WRN SMARCA4 RAD51
Cyclin binding	CDKN1A
Catalytic activity	XRCC5 ERBB2 XRCC6 PRKDC WRN CDK4 STUB1 MMP2 SIRT1 ATM RAD51 CDKN1A PCNA MAPK8 HDAC9 SMARCA4 BARD1
1-phosphatidylinositol-3-kinase activity	ATM
NAD binding	SIRT1
Phosphoinositide 3-kinase activity	ATM
Death receptor binding	BID
Cyclin-dependent protein kinase inhibitor activity	CDKN1A
Unfolded protein binding	HSP90AA1 NPM1
Promoter binding	XRCC5 XRCC6
Hydrolase activity, acting on carbon-nitrogen (but not	HDAC9 SIRT1
DNA N-glycosylase activity	PCNA
Caspase inhibitor activity	BCL2L1
DNA regulatory region binding	XRCC5 XRCC6

(D) Cluster 4

Molecular Function	Genes
DNA-dependent ATPase activity	CKN2 ERCC3 ERCC2
Protein N-terminus binding	CKN2 ERCC3 ERCC2
Protein C-terminus binding	CKN2 ERCC3 ERCC2
ATP-dependent DNA helicase activity	ERCC3 ERCC2
ATPase activity, coupled	CKN2 ERCC3 ERCC2
DNA helicase activity	ERCC3 ERCC2
Damaged DNA binding	DDB2 ERCC3
ATPase activity	CKN2 ERCC3 ERCC2
dATP binding	ERCC3
DNA binding	CKN2 DDB2 CDK9 TBP ERCC3
ATP-dependent helicase activity	ERCC3 ERCC2
Purine NTP-dependent helicase activity	ERCC3 ERCC2
Purine deoxyribonucleotide binding	ERCC3
Adenyl deoxyribonucleotide binding	ERCC3
ATP binding	CKN2 CDK9 ERCC3 ERCC2
Adenyl ribonucleotide binding	CKN2 CDK9 ERCC3 ERCC2
Deoxyribonucleotide binding	ERCC3
Helicase activity	ERCC3 ERCC2
Adenyl nucleotide binding	CKN2 CDK9 ERCC3 ERCC2
Purine nucleoside binding	CKN2 CDK9 ERCC3 ERCC2
Nucleoside binding	CKN2 CDK9 ERCC3 ERCC2
RNA polymerase II carboxy-terminal domain kinase activity	CDK9
Nucleoside-triphosphatase activity	CKN2 ERCC3 ERCC2
Pyrophosphatase activity	CKN2 ERCC3 ERCC2
Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	CKN2 ERCC3 ERCC2
Hydrolase activity, acting on acid anhydrides	CKN2 ERCC3 ERCC2
5′-3′ DNA helicase activity	ERCC2
3′-5′ DNA helicase activity	ERCC3
Nucleic acid binding	CKN2 DDB2 CDK9 TBP ERCC3
Purine ribonucleotide binding	CKN2 CDK9 ERCC3 ERCC2
Ribonucleotide binding	CKN2 CDK9 ERCC3 ERCC2
Purine nucleotide binding	CKN2 CDK9 ERCC3 ERCC2
snRNA binding	CDK9
Nucleotide binding	CKN2 CDK9 ERCC3 ERCC2
Transcription elongation regulator activity	CKN2
Cyclin-dependent protein kinase activity	CDK9
Transcription factor binding	TBP ERCC3
General RNA polymerase II transcription factor activity	TBP
Iron-sulfur cluster binding	ERCC2
Metal cluster binding	ERCC2
Protein binding	CKN2 DDB2 CDK9 TBP ERCC3 ERCC2

(E) Cluster 5

Molecular function	Genes
Transcription regulator activity	SIN3B SIN3A HDAC2 TRIM28 NFYC NFYB NFYA MYB CARM1 ING1
Transcription activator activity	HDAC2 TRIM28 NFYC NFYA MYB CARM1 ING1
Transcription factor binding	SIN3B SIN3A HDAC2 TRIM28 NFYC CARM1
Transcription cofactor activity	SIN3B SIN3A TRIM28 NFYC CARM1
Transcription repressor activity	SIN3B SIN3A HDAC2 TRIM28
Transcription corepressor activity	SIN3B SIN3A TRIM28
DNA binding	SIN3B SIN3A HDAC2 NFYC NFYB NFYA MYB
Transcription coactivator activity	TRIM28 NFYC CARM1
Nucleic acid binding	SIN3B SIN3A HDAC2 NFYC NFYB NFYA MYB
Protein binding	SIN3B SIN3A HDAC2 TRIM28 NFYC NFYB NFYA MYB CARM1 ING1
Transcription factor activity	SIN3A NFYC NFYB NFYA
Histone-arginine N-methyltransferase activity	CARM1
Chromo shadow domain binding	TRIM28
Arginine N-methyltransferase activity	CARM1
Protein-arginine N-methyltransferase activity	CARM1
Sequence-specific DNA binding	HDAC2 NFYC NFYB
Transcription repressor binding	NFYB
Histone deacetylase activity	HDAC2
Protein deacetylase activity	HDAC2
Deacetylase activity	HDAC2

(F) Cluster 6

Molecular function	Genes
Protein-arginine omega-N symmetric methyltransferase activity	PRMT5
Histone-arginine N-methyltransferase activity	PRMT5
Arginine N-methyltransferase activity	PRMT5
Protein-arginine N-methyltransferase activity	PRMT5
Transcription repressor binding	KAT5
Telomeric DNA binding	NCL
Androgen receptor binding	KAT5
Histone methyltransferase activity	PRMT5
Ribonucleoprotein binding	PRMT5
Steroid hormone receptor binding	KAT5
Protein methyltransferase activity	PRMT5
N-methyltransferase activity	PRMT5

(G) Cluster 7

Molecular function	Genes
Protein-arginine omega-N symmetric methyltransferase activity	PRMT5
Histone-arginine N-methyltransferase activity	PRMT5
Arginine N-methyltransferase activity	PRMT5
Protein-arginine N-methyltransferase activity	PRMT5
Transcription repressor binding	KAT5
Telomeric DNA binding	NCL
Androgen receptor binding	KAT5
Histone methyltransferase activity	PRMT5
Ribonucleoprotein binding	PRMT5
Steroid hormone receptor binding	KAT5
Protein methyltransferase activity	PRMT5
N-methyltransferase activity	PRMT5

(H) Cluster 8

Molecular function	Genes
Double-stranded RNA binding	RNASEN DGCR8
RNA binding	RNASEN DGCR8 DDX5
Ribonuclease III activity	RNASEN
Endoribonuclease activity, producing 5'-phosphomonoesters	RNASEN
RNA helicase activity	DDX5
Endonuclease activity, active with either ribo- or deoxyribonucleic acids and producing 5' phosphate	RNASEN
Endoribonuclease activity	RNASEN
Nucleic acid binding	RNASEN DGCR8 DDX5

bone marrow angiogenesis through binding to VEGF receptors. EGFR is an oncogene homolog and acts receptor for EGF and other members of the EGF family including TGF-alpha, amphiregulin, betacellulin, heparin-binding EGF-like growth factor, GP30 and vaccinia virus g rowth factor. Furthermore, it also involved in the cell growth control and differentiation. Third cluster comprises 34 proteins that functionally active in binding of protein, DNA, death receptor, ATP, BH3 domain; and plays crucial role in cell cycle, programmed cell-death, transcription repressor activity and organelle organization. This gene encodes a transcription factor that involves in apoptosis in response to cellular stress, stimulus and DNA damage.

Two vital genes (BCL2 and BID) involved in this cluster. BCL2 protein is involved in regulation of cell death through controlling mitoch ondrial membrane permeability.

It plays an important role in a feedback loop system with caspases. It inhibits caspase activity either via preventing the release of cytochrome c from the mitochondrio or through binding to the apoptosis-activating factor (APAF1). BID counters the protective effect of BCL2. The major proteolytic product p15BID allows the cytochrome c release which is important for caspase activity.

In forth cluster, the proteins were mainly involved in cell cycle, cell proliferation, apoptosis and regulation of transcription. This protein influence cell cycle arrest and programmed in response to cellular stress, ionizing radiation, toxin and DNA damage. However, the proteins in fifth cluster were actively participated in regulation of transcription, RNA metabolic process, macromolecule biosynthetic process, nucleobase, nucleosides, nucleotides and nucleic acid metabolic process and regulation of nitrogen compound metabolic process.

On the other hand, the proteins in sixth cluster normally play key roles in organelle organization, chromatin organization and chromosome organization, chromatin assembly and disassembly, histone modification and covalent chromatin modification whereas proteins in cluster 7 mainly involved in induction of apoptosis by extracellular signals, membrane protein intracellular domain proteolysis, membrane protein proteolysis, regulation of cell differentiation and developmental process. Finally, cluster 8 associated with proteins usually participated in gene silencing by RNA, RNA processing, RNA metabolic process and rRNA catabolic process.

Protein-protein interactions play a key role in different types of biological processes such as cell cycle, metabolic pathways and signal transduction (18-19). Therefore, studying these protein-protein interactions is notable. This is because it can reveal information about the regulation of cellular activities.

Ciriello and his colleagues (2013) derived a hierarchical classification of 3,299 TCGA (20) tumors from twelve different types of cancer. The top classes are dominated by either mutations (M class) or alteration of copy number (C class). This significance is clearest at the extremes of genomic instability, which shows the presence of different oncogenic processes. The hierarchy describes the targetable functional events in tumor. Based on their study, TP53 mutations were strongly enriched in the C class. These mutations causing copy number genomic instability. Breast, ovarian, lung squamous cell, head and neck squamous cancer and endometrioid tumors of the serous subtype are included in C class. The C class subdivided into two groups, primarily determined by the absence (subclasses C1-C6) or presence (subclasses C7-C14) of gains and losses on chromosome 8.

A major portion of lung cancer consists of subclass C3 while subclass C4 included a large fraction of head and neck squamous cancer. This proved as an example of cross-cancer similarity, in which alterations of genomics are shared by subsets of tumors of different origin. In lung cancer, subclass C3 was classified by mutation of TP53, copy number amplification of 3q26 and deletion of CDKN2A; whereas subclass C4 in head and neck squamous cancer had focal chromosomal copy number amplification of 11q13 where CCND1 is located. Some of these genomic differences actually converged on the same pathway, as loss of CDKN2A (C3) and gain of

CCND1 (C4) both impair Rb-mediated cell cycle control. In breast and ovarian cancer, alteration of copy number in subclasses C7-C14 affects the cell cycle regulation and DNA damage response pathways. The G1/S phase checkpoint was compromised by amplification of CCNE1 in subclasses C7 and C11. Then, it was bypassed by amplification of E2F3 in subclass C13. Inactivation of BRCA1 and BRCA2 genes were caused by defective cell cycle arrest and DNA damage in subclass C13. Lastly, amplification and overexpression of the regulator of mitosis AURKA was occurred in subclass C14 (21-22).

Alterations in the protein of p53 pathway might cause disruption of the pathway and thus might be significant contributors to the tumorigenesis process. For example, the blockade of the cell cycle and PI3K-AKT signalling in lung and head and neck squamous cell cancer; and inhibition of PARP and AURKA which causes the inactivation of BRCA1 or BRCA2 in breast and ovarian cancer prevent the proliferation of cancer cells and the abnormal cells undergo apoptosis.

Conclusion

In this study, the TP53 protein network analysis proved that the core regulation of p53 was stabilized via its interaction with several proteins. However, the proteins were functionally interacted with each other so alteration of protein might disrupt expression of interacting proteins and leads to the pathogenesis of disease. Exploring about gene expression in TP53 protein network could aid in identifying new pathways of disease pathogenesis. Besides that, a better understanding of the relationship between the genes could aid in developing prognostic markers and better therapeutic strategies in breast cancer treatment.

Conflict of interests

All authors of this publication declare that there are no conflicts of interest in publishing this research article.

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List of Abbreviation

DNA	- Deoxyribonucleic acid
PI3K	- Phosphatidylinositol-4,5-bisphosphate 3-kinase
AKT	- Protein kinase B
MAPK	- Mitogen-activated protein kinase
PARP	- Poly (ADP-ribose) polymerase
TP53	-Tumor suppressor gene 53
BCL2	- B-cell lymphoma 2
MDM2	- Mouse double minute 2 homolog
BAX	- Bcl-2-associated X protein
HER2	- Human epidermal growth factor receptor 2
PTEN	- Phosphatase and tension homolog deleted on chromosome ten
p21	- Cyclin-dependent kinase inhibitor 1
NLS	- Nuclear localization signal
CDK2	-Cyclin dependent kinase 2
BRCA1	- Breast cancer type 1 susceptibility protein
BRCA2	- Breast cancer type 2 susceptibility protein
CASP3	-Caspase 3
NCID	-Notch intracellular domain
PDGF	-Platelet-derived growth factor
EGF	-Epidermal growth factor
IGF-I	-Insulin and insulin-like growth factor I