Mapping of TP53 protein network using Cytoscape software

Asita Elengoe1, Salehuddin Hamdan2

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

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 tumorogenesis 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 1.3.2 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.

Citation: Pharm Biomed Res 2018; 4(3): 9-25.

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 ulcers, 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 tumorigenesis 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 a 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 promoters of tumorigenesis 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; probabilistic 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 tumorigenesis 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 the 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 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 organic form.
The effects of various vascular permeability and the enhancer of split locus. It hinders the differentiation through activation of ligand. Then, it activates a transcriptional activator complex with RBP determination. Notch intracellular domain (NCID) forms ligands Jagged1, Jagged2 and Delta1 to regulate cell determination. Notch1 plays a 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, cyclic 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 1 (IGF-1). 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...
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, CKD1, TP53, BLM, Y11, SKP2, BRCA1, CCND1, MAX, NOTCH1, PKR, HRA5, CKD2A, MAPK1, MAPK14, ESR1, PTEF, AURKA, AR, MDM2, MLH1, IDH1, BAX, BIRC5, PTSG2, HIP1A, SMAD2, KAT2B, STAT3, CCN1I, CCN2, TGBF1, BRCA2 | 3.771 | 35 | 132 |
| 2       | CREBBP, AKT1, EP300, RB1, MNAT1, CKD2, CKD25C, CHEK1, CCNB1, CDC25A, MYC, SFP, JUN, P53, VE1GA, IGF1, IL6, IGF2, TGFB2 | 3.474 | 19 | 66 |
| 3       | CDK9, BAK1, BCL2L11, WRN, PMI1P1, XRC5, HSP90AA1, XRC6, BCL2, MCL1, BCC3, RAD51, STUB1, BDFB4, CCNA2, ING2, ERBB2, BARD1, MAPK8, PLA2, PMA, P11, MPP2, SMACG4, NF1, UBC, CKD4, ATM, HDAC9, PRKD1, HSPAA, SRT1, DAXX, BID, GADD45A | 2.941 | 34 | 100 |
| 4       | CKN2, ERC3, CDX9, ERG2, TBP, DDB2 | 1.500 | 6 | 9 |
| 5       | NFXL, NFXB, NFYA, CARM1, SIN3A, TRIM38, MYC, JUN, FOS, VEGFA, IGF1 | 1.300 | 10 | 13 |
| 6       | NCL, H3F3A, KAT5, PIM5 | 1.000 | 4 | 4 |
| 7       | MAPK9, NGR, 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, BHC5, SMAD2, PTFEN, BRCA1, STAT3, MAPK1, CCND1, HIP1A, CDKN2A, HDAC1, PLK1, MDM2 |
| Transcription factor binding | AR, KAT2B, CREB1, Y11, TP53, ESR1, SMAD2, CKD7, BRCA1, STAT3, MAPK1, MAX, HIP1A, CDKN2A, HDAC1 |
| Transcription activator activity | MAX, NOTCH1, AR, KAT2B, HDAC1, Y11, TP53, BRCA2, SMAD2, CKD7, STAT3, BRCA1, TGFB1 |
| Protein binding    | HRS5, BLM, PTG2, MLH1, AURKA, PTFEN, TGFB1, MAX, CDKN2A, CD44, CD11, AR, KAT2B, CCN6, CREB1, Y11, TP53, SKP2, ESR1, BRCA2, BIRC5, SMAD2, CDK7, PKR, STAT3, BRCA1, MAPK1, CCND1, NOTCH1, HIP1A, HDAC1, PLK1, MDM2 |
| Protein kinase binding | MAPK1, CCND1, KAT2B, CDKN2A, PLK1, TP53, AURKA, STAT3 |
| Transcription regulator activity | AR, KAT2B, CREB1, Y11, TP53, ESR1, BRCA2, SMAD2, CDK7, STAT3, TGFB1, BRCA1, MAX, NOTCH1, HIP1A, 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, Y11, ESR1, TP53, SMAD2 |
| DNA regulatory region binding | MAX, AR, Y11, ESR1, TP53, SMAD2 |
| Sequence-specific DNA binding | MAX, NOTCH1, AR, HIP1A, Y11, CREB1, ESR1, TP53, SMAD2, STAT3 |
| Receptor signalling protein activity | MAPK1, CD44, PLK1, MAPK14, CREB1, SMAD2, STAT3 |
| Double-stranded DNA binding | BLM, CREB1, TP53, MLH1, SMAD2 |
| DNA binding        | AR, BLM, CREB1, Y11, TP53, ESR1, MLH1, BRCA2, SMAD2, STAT3, BRCA1, MAPK1, MAX, NOTCH1, HIP1A, CDKN2A, HDAC1 |
| Protein dimerisation activity | MAX, AR, HIP1A, BAX, CREB1, TP53, BIRC5, STAT3, TGFB1 |
| Transcription factor activity | MAX, NOTCH1, AR, HIP1A, HDAC1, Y11, CREB1, ESR1, TP53, SMAD2, STAT3 |
| Protein heterodimerisation activity | MAX, HIP1A, BAX, TP53, BIRC5, TGFB1 |
| Cyclin-dependent protein kinase activity | CCND1, KAT2B, CDKN2A |
| Ubiquitin protein ligase activity | TP53, SMAD2, AURKA, BRCA1 |
| p33 binding       | CDKN2A, BLM, MDM2 |
| DNA strand annealing activity | BLM, TP53 |

Pharm Biomed Res 2018; 4(3): 14
| Functional Category | Associated Proteins |
|---------------------|---------------------|
| RNA polymerase II carboxy-terminal domain kinase activity | CDK1 CDK7 |
| Protein kinase activity | MAPK1 CDK1 CCND1 PLK1 MAPK14 AURKA PRR CDK7 |
| Protein serine/threonine kinase activity | MAPK1 CDK1 PLK1 MAPK14 AURKA PRR CDK7 |
| Phosphotransferase activity, alcohol group as acceptor | MAPK1 CDK1 CCND1 PLK1 MAPK14 AURKA PRR CDK7 |
| Transcription coactivator activity | MAX KAT2B YY1 CDK7 BRCA1 |
| Transcription cofactor activity | MAX KAT2B YY1 CREB1 CDK7 BRCA1 |
| Protein complex binding | MAX CCND1 PLK1 ESRR1 CDK7 |
| Kinase activity | MAPK1 CDK1 CCND1 PLK1 MAPK14 AURKA PRR CDK7 |
| Single-stranded DNA binding | BLM TP53 BRCA2 |
| Nucleic acid binding | AR BLM CREB1 YY1 TP53 ESRR1 MLH1 BRCA2 SMAD2 STAT3 BRCA1 MAPK1 MAX NOTCH1 HIF1A CDKN2A |
| 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 PTE1 TGFBI1 MAX CDKN2A CD44 CDK1 AR KAT2B CCND1 CREB1 YY1 TP53 ESRR1 BRCA2 BIRC5 SMAD2 STAT3 BRCA1 MAPK1 CCND1 NOTCH1 HIF1A HDM1 PLK1 MAPK14 BAX MDM2 MAPK1 |
| MAP kinase activity | MAPK1 |
| Transferase activity, transferring phosphorus-containing groups | MAPK1 CDK1 CCND1 PLK1 MAPK14 AURKA PRR CDK7 |
| - Transforming growth factor-beta receptor binding | SMA2 TGFBI1 |
| 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 PRR CDK7 |
| Adenyl ribonucleotide binding | MAPK1 CDK1 BLM PLK1 MAPK14 TP53 MLH1 AURKA PRR CDK7 |
| Ribonucleotide binding | CCND1 KAT2B CDKN2A |
| Tubulin binding | BRCA2 BIRC5 BRCA1 |
| Protease binding | TP53 BRCA2 |
| Purine ribonucleotide binding | MAPK1 CDK1 HRAS BLM PLK1 MAPK14 TP53 MLH1 AURKA PRR CDK7 |
| Ribonucleotide binding | MAPK1 CDK1 HRAS BLM PLK1 MAPK14 TP53 MLH1 AURKA PRR CDK7 |
| Cyclin-dependent protein kinase activity | CDK1 CDK7 |
| Adenyl nucleotide binding | MAPK1 CDK1 BLM PLK1 MAPK14 TP53 MLH1 AURKA PRR CDK7 |
| Androgen receptor binding | CDK7 BRCA1 |
| Chaperone binding | TP53 BIRC5 |
| Lipid binding | AR PTGS2 BAX ESRR1 PTE1 |
| POU domain binding | AR |
| Estrogen response element binding | ESRR1 |
| MAP kinase 2 activity | MAPK1 |
| Insulin-like growth factor 1-3,4,5-tetrakisphosphate 3-phosphatase activity | PTEN |
| Phosphatidylinositol-3,4,5-trisphosphate 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 PRR CDK7 |
| Nucleoside binding | MAPK1 CDK1 BLM PLK1 MAPK14 TP53 MLH1 AURKA PRR CDK7 |
| Purine nucleotide binding | MAPK1 CDK1 HRAS BLM PLK1 MAPK14 TP53 MLH1 AURKA PRR CDK7 |
| Protein kinase inhibitor activity | KAT2B CDKN2A |
| Enhancer binding | HIF1A CREB1 |
| RNA polymerase II transcription factor activity, enhancer binding | HIF1A CREB1 |

**References:**

Elengoe et al. 2018; 4(3): 15

**Journal:** Pharm Biomed Res

**Volume:** 2018

**Issue:** 4(3)

**Pages:** 15
| Activity Type                                      | Protein(s)          |
|--------------------------------------------------|---------------------|
| Specific transcriptional repressor activity      | HDAC1 YY1           |
| Kinase inhibitor activity                        | KAT2B CDKN2A        |
| Histone deacetylase binding                      | KAT2B H4AC1         |
| Identical protein binding                        | HDAC1 BAX MDM2 BIRC5 BRCA1 TGFBI |
| 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 PBR CDK7 |
| Androgen binding                                 | AR                  |
| ATP-dependent 3'-5' DNA helicase activity         | BLM                 |
| Ubiquitin-protein ligase regulator activity      | CDKN2A              |
| Ligase regulator activity                        | CDKN2A              |
| Transferase activity                             | MAPK1 CDK1 CCND1 KAT2B PLK1 MAPK14 AURKA PBR CDK7 |
| Steroid binding                                  | AR ESR1             |
| Transforming growth factor beta receptor, pathway-specific cytoplasmic mediator activity | SMAD2               |
| Homologous/histocompatibility 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 HSF1A          |
| Protein N-terminus binding                       | TP53 TGFBI          |
| Death domain binding                             | BAX                 |
### Mapping of TP53 protein network

| Molecular Function | Genes |
|--------------------|-------|
| SAP kinase activity | MAPK14 |
| Hsp70 protein binding | CDK1 |
| Nuclear hormone receptor binding | CDK7 BRCA1 |
| Type II transforming growth factor beta receptor binding | TGFBI |
| Type I transforming growth factor beta receptor binding | SMAD2 |
| Histone kinase activity | CDK1 |
| Receptor binding | AR SMAD2 CDK7 PTEN BRCA1 TGFBI |
| 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 bisphosphate 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 CKK2A 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 I6L CREBBP TGFI CHEK1 RB1 CDC25C CKIK2 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 |
| MRLH 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 |
| Similar protein binding          | AKT1 EGFR SP1 VEGFA TOP2A CDK2 |
|---------------------------------|--------------------------------|
| Histone acetyltransferase 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                        |
| Transferrase 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 activity   | EP300 JUN CREBBP RB1                |
| Transferrase 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 topoisomerase (ATP-hydrolysing) activity | TOP2A |
| Hormone receptor binding        | EP300 RB1                           |
| Nitric-oxide synthase regulator activity | AKT1 |
| E-box binding                   | MYC                                 |
| Protein tyrosine phosphatase activity | CDC25 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                               |
### Molecular function

| Genes | Functions |
|-------|-----------|
| EGFR, FOS, EP300, SP1 | Nucleic acid binding |
| SP1, VEGFA, TOP2A | Protein homodimerisation activity |
| SP1 | Histone acetyltransferase binding |
| VEGFA | Fibronectin binding |
| Akt1 | Phosphatidylinositol-3,4-biphosphate binding |
| SP1, TOP2A | Protein C-terminus binding |
| EGFR, IL6, CREBBP, Igf1, Chek1, BBI, CDC25C, CDK2, CDC25A, CCNB1, AKT1, FOS, MNAT1, EP300, SP1, JUN, VEGFA, MYC, TOP2A | Binding |
| VEGFA | Insulin-like growth factor binding |
| CDC25C, CDC25A | Phosphoprotein phosphatase activity |
| VEGFA | Chemoattractant activity |
| EGFR | Receptor signalling protein tyrosine kinase activity |
| CREBBP, TOP2A | Chromatin binding |
| Akt1 | Phosphatidylinositol-3,4,5-triphosphate binding |
| EP300, CREBBP | Acetyltransferase activity |
| EP300, CREBBP | Transferase activity, transferring acyl groups other than amino-acyl groups |
| IL6, VEGFA | Cytokine receptor binding |
| VEGFA | Insulin-like growth factor binding |
| CDC25C | Phosphoprotein phosphatase activity |
| CDC25C, CDC25A | Chemoattractant activity |
| EGFR | Receptor signalling protein tyrosine kinase activity |
| VEGFA | Protein C-terminus binding |
| CDC25C | Chromatin binding |
| CDC25A | Phosphatidylinositol-3,4,5-triphosphate binding |
| Akt1 | Acetyltransferase activity |
| CREBBP | Transferase activity, transferring acyl groups |
| CDC25C | MAP kinase activity |
| CDC25A | WW domain binding |
| CDC25C | Cytokine activity |

### (C) Cluster 3

| Genes | Functions |
|-------|-----------|
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | DNA-dependent ATPase activity |
| XRCC6 | Protein homodimerisation activity |
| XRCC6 | ATP binding |
| XRCC6, XRCC8 | Enzyme binding |
| XRCC6, XRCC8 | Histone binding |
| XRCC6 | Adenyl ribonucleotide binding |
| XRCC6 | Adenyl nucleotide binding |
| XRCC6 | Structure-specific DNA binding |
| XRCC6 | Purine nucleoside binding |
| XRCC6 | DNA-dependent protein kinase activity |
| XRCC6 | TPR domain binding |
| XRCC6 | Nucleoside binding |
| XRCC6 | Protein C-terminus binding |
| XRCC6 | p53 binding |
| XRCC6 | ATP-dependent DNA helicase activity |
| XRCC6 | DNA repair domain binding |

### Table: Molecular function

| Genes | Functions |
|-------|-----------|
| HSP90AA1, ERBB2, PML, BCL2, WRN, STUB1, DAXX, SIRT1, ATM, RAD51, BAK1, BCL2, NPM1, SMARC4, BARD1 | Identical protein binding |
| BAK1, HSP90AA1, MCL1, BCL2, ERBB2, NPM1, PML, WRN, BCL2, DAXX, STUB1, ATM, BARD1 | Protein dimerisation activity |
| BAK1, MCL1, BCL2, ERBB2, NPM1, PML, BCL2, L1, BARD1 | Protein heterodimerisation activity |
| BID, XRCC5, ING2, MCL1, ERBB2, XRC6, PML, PRKDC, PAAAP1, BCL2, L1, MMP2, STUB1, DAXX, BAK1, BCL2, NPM1, CCNA2, BBI, HSRPAA1, WRN, CDK4, SIRT1, ATM, RAD51, CDKN1A, HBC3, PCNA, MAPK8, HDAC9, GADD45A, SMARC4, BARD1 | Protein binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | DNA-dependent ATPase activity |
| HSP90AA1, BCL2, NPM1, PML, WRN, DAXX, STUB1, BARD1 | ATP binding |
| BAK1, HSP90AA1, MCL1, BCL2, ERBB2, NPM1, PML, WRN, BCL2, L1, BARD1 | Enzyme binding |
| BAK1, HSP90AA1, MCL1, BCL2, ERBB2, NPM1, PML, WRN, BCL2, L1, BARD1 | Histone binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | Adenyl ribonucleotide binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | Adenyl nucleotide binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | Structure-specific DNA binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | Purine nucleoside binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | DNA-dependent protein kinase activity |
| HSP90AA1, STUB1 | TPR domain binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | Nucleoside binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | Protein C-terminus binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | p53 binding |
| XRCC5, XRCC6, WRN, SMARC4, RAD51 | ATP-dependent DNA helicase activity |
| BCL2, BCL2L1 | DNA repair domain binding |
| Protein Function                                      | Proteins                                                                 |
|-------------------------------------------------------|----------------------------------------------------------------------------|
| **Double-stranded DNA binding**                       | XRCC5, XRCC6, PCNA, RAD51                                                 |
| **Purine ribonucleotide binding**                      | XRCC5, HSP90AA1, ERBB2, XRCC6, PRKDC, WRN, CDK4, ATM, RAD51, CKDN1A, HSPA4, MAPK8, SMARCA4 |
| **Ribonucleotide binding**                            | XRCC5, HSP90AA1, ERBB2, XRCC6, PRKDC, WRN, CDK4, ATM, RAD51, CKDN1A, HSPA4, MAPK8, SMARCA4 |
| **DNA helicase activity**                             | XRCC5, XRCC6, WRN                                                         |
| **Purine nucleotide binding**                         | XRCC5, HSP90AA1, ERBB2, XRCC6, PRKDC, WRN, CDK4, ATM, RAD51, CKDN1A, 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, CKDN1A, HSPA4, MAPK8, SMARCA4 |
| **Protein complex binding**                           | CKDN1A, ING2, PCNA, WRN, ATM                                             |
| **Protein domain specific binding**                   | CKDN1A, 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                                    |
| **Ubiquitin 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-terminal binding**                        | DAXX, ATM, SMARCA4                                                       |
| **Binding**                                            | BID, XRCC5, ING2, MCL1, ERBB2, XRCC6, PML, PRKDC, PMAIP1, BCL2L1, MMP2, STUB1, DAXX, BAK1, BCL2, NPM1, HSPA4, CCND2, RBBP4, HSP90AA1, WRN, CDK4, SIRT1, ATM, RAD51, CKDN1A, BRC3, PCNA, MAPK8, HDAC9, GADD45A, SMARCA4, BARD1 |
| **Deacetylase activity**                              | HDAC9, SIRT1                                                              |
| **Protein kinase activity**                           | CKDN1A, 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**          | CKDN1A, 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                                                                     |
| **DNA binding**                                       | XRCC5, ING2, BCL2, ERBB2, XRCC6, PCNA, PML, PRKDC, WRN, ATM, SMARCA4, RAD51 |
| **Phosphotransferase activity, alcohol group as acceptor** | CKDN1A, ERBB2, PRKDC, MAPK8, CDK4, ATM                                  |
| **Histone deacetylase binding**                       | RRBP4, 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                                                                     |
| **DNA polymerase processivity factor activity**       | PCNA                                                                     |
| **DNA binding**                                       | XRCC5, ING2, BCL2, ERBB2, XRCC6, PCNA, PML, PRKDC, WRN, ATM, SMARCA4, RAD51 |
| **Histone deacetylase activity**                      | RRBP4, 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                                                                      |
| **Histone deacetylase activity**                      | RRBP4, 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                                                                      |
| **Histone deacetylase activity**                      | RRBP4, HDAC9                                                              |
| **Transcription regulator activity**                  | ING2, BCL2, NPM1, XRCC6, PML, HDAC9, SIRT1, DAXX, SMARCA4                |
| Activity                                                                 | Protein(s) |
|------------------------------------------------------------------------|------------|
| NAD-dependent histone deacetylase activity                            | SIRT1      |
| NAD-dependent protein deacetylase activity                            | SIRT1      |
| Cobalt ion binding                                                    | PML        |
| Single-stranded DNA-dependent ATPase activity                         | RAD51      |
| JNK kinase activity                                                    | MAPK8      |
| McLa alpha complex binding                                            | PCNA       |
| Y-form DNA binding                                                    | WRN        |
| G-quadruplex DNA binding                                              | WRN        |
| Ribosomal large subunit binding                                       | NPM1       |
| Hydrolyase activity, acting on carbon-nitrogen                        | HDAC9 SIRT1|
| Transcription coactivator 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 |
| Transleasease activity, transferring phosphorus-containing histidine | 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 |
| Hydrolyase activity, acting on acid anhydrides                        | XRCC5 XRCC6 WRN SMARCA4 RAD51 |
| Hydrolyase activity, acting on acid anhydrides                        | XRCC5 XRCC6 WRN SMARCA4 RAD51 |
| Cyclin binding                                                       | CDKN1A     |
| Catalytic activity                                                    | XRCC5 ERBB2 XRG6 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 |
| Hydrolyase 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 nucleotide 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 |
| 3'-5' 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 NFYB 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 |
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 growth 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 mitochondrial 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, chromat 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.

**References**

1. World Health Organization (WHO) (2017): Available from: http://www.who.int/mediacentre/factsheets/fs297/en/. (cited: 7th Sept 2017).

2. Hawkins LK, Hermiston, T. Gene delivery from the E3 region of replicating human adenovirus evaluation of the E3B region. Gene Ther 2001;8:1142-8.

3. Vermeulen K, van Buckstaele DR, Berneman ZN. The cell cycle: A review of regulation, deregulation and therapeutic targets in cancer. Curr Pharm 2003;36:131-49.

4. Ichimura K, Bollin MB, Golke HM, Schmidt EE, Moshref A. Deregulation of the p14ARF/MDM2/p53 pathway is a prerequisite for human astrocytic gliomas with G1-S transition control gene abnormalities. Cancer Res 2000;60:417-24.

5. Gustafsson B, Akesson B, Gustafsson B, Chrestenson B, Winiarski J. MDM2 and p53 in childhood acute lymphoblastic leukemia: Higher expression in childhood leukemias with poor prognosis compared to long-term survivors. Pediatr Hematol Oncol 2001;18:497-508.

6. Peller S, Rotter V. TP53 in hematological cancer: Low incidence of mutations with significant clinical relevance. Hum Mutat 2003;21:277-84.

7. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1990;90:405-13.

8. Liu X, Kim CN, Yang J, Jemmerson R, Wang, X. Induction of apoptotic program in cell-free extracts: Requirement for dATP and cytochrome c. Cell 1996;86:47-57.

9. Wachi S, Yoneda K, Wu R. Interactome-transcriptome analysis reveals the high centrality of genes differentially expressed in lung cancer tissues. Bioinformatics 2005;21:420-6.

10. Goehler H, Lalloowski M, Stehl U, Waater S, Stroedicke M. A protein interaction network links G1T1, an enhancer of huntingtin aggregation, to Huntington’s disease. Mol Cell 2004;15:853-65.

11. Jonsson P, Bates PA. Global topological features of cancer proteins in the human interactome. Bioinformatics 2006;22:2291-7.

12. Jayaraman A, Jamil K, Raju S. The interaction of p53 and MDM2 genes in cancers, in silico studies and phylogenetic analysis. Bio Med. 2011;3:1-12.

13. Wagner A. How the global structure of protein interaction networks evolves. Proc R Soc Lond 2003;270:457-66.

14. Veselovsky AV, Ivanov YD, Ivanov AS, Archakov AJI. Protein-protein interactions mechanisms and modifications by drugs Mol Rec 2002;15:405-22.

15. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A. The STRING database in 2011: Functional interaction networks of proteins, globally integrated and scores. Nucleic acids Res 32;2011:561-8.

16. Jayaraman A, Jamil K. Mapping the p53 gene using STRING software to study the alterations modulating the functioning of associated genes in leukemia. Indian J Biotechnol 2013;12:451-61.

17. Lin C, Cho YK, Hwang WC, Pei PI, Zhang AD. Clustering methods in protein-protein interaction network. Knowledge Discovery in Bioinformatics Techniques, Methods and Application 2006; 1-35.

18. Pawson T, Nash, P. Protein-protein interactions define specificity in signal transduction. Genes Develop 2000;14:1027-47.

19. Ozgur A, Yu T, Erik C, Gadev DR. Identifying gene-disease associations using centrality on a literature mined gene-interaction network. Bioinform 2008;24:277-85.

20. Costello G, Miller, ML, Aksay BA, Semba H, Itzhak Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. Nature genetics 2013;45:1127-33.

21. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumors. Nature 2012;490:61-70.

22. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609-15.