SNP Mutation-related Genes in Colon Cancer for Monitoring and Prognosis of Patients: A Study Based on the TCGA Database

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Abstract

Background: Cancer is still the leading cause of death in humans, and the fourth leading cause of death is colorectal cancer. Tumor bioinformatics has been developing in recent years, the prognosis and quality of life of patients can be improved by using relevant tools to understand the molecular pathogenesis of colorectal cancer and related prognostic markers.

Methods: In this study, Bioinformatics analysis of the snp-related data of colon cancer patients from the TCGA database, it was found that the expression levels of 4 mutated genes (CTTNBP2,DAPK1, DMXL1,SPTBN2) were significantly different from those of wild type and their prognosis. In order to explore how the core genes affect the prognosis of patients, the gene expression of these core genes was analyzed.

Results: It was found that the core genes are related to a variety of cancer-related pathway genes, including pi3k-akt pathway and TSC/mTOR pathway. Drug sensitivity analysis showed that SPTBN2 could be inhibited by a variety of drugs, including austocystin D, afatinib, and belinostat. Tumor immunity is closely related to tumor therapy. Through the analysis of immune infiltration of core genes, it was found that DAPK1 and DMXL2 were associated with a variety of immune cell infiltration.

Conclusion: Therefore, the detection of genetic mutations and related expressions may be significant in predicting the prognosis of patients with colon cancer. Through the study of high-throughput information excavating, it was discovered that the molecular pathogenesis and prognosis of patients with colon cancer were helpful to the bioinformatics theory.

Background

Nowadays, the number of people dying from cancer continues to increase worldwide. For example, about 17.2 million people worldwide suffered from cancer in 2016, with about 890,000 deaths reported in Global burden of Disease study (1). Colorectal cancer is the third most familiar cancer in the world, and it's a cause for concern and the fourth leading cause of cancer deaths (2). According to previous studies, we can know that this serious disease involves many changes in genomics, genetics, and epigenetics. The molecular mechanism of level development process can help us reveal the occurrence of colon cancer individual accurate treatment. As we know, single nucleotide aberrance causes single nucleotide polymorphisms (SNPs), also known as DNA array polymorphisms. Moreover, in the mankind genome, it is the most familiar type of mutation (3). It is a remarkable fact that the SNP located in different location has different functions. But the happening of the disease has a close connection with rRNA and miR-rRNA which locate in protein-coding and non-coding genes initiator. And it can alter the expression of genes. For example–125G/A rSNP of BAX gene, which encodes pro-apoptotic protein BAX (a tumor-suppressor gene): Compared with the G allele, the A allele leads to decreased mRNA-protein level expression, there is also an increased risk of chronic lymphocytic leukemia (4). srSNP in pre-mRNA and mature mRNA can change the stability, length, and transcription of mRNA/miRNA. The mutated

individuals of srSNPs located in the 5’ UTR of protein-coding genes were associated with platinum drug resistance (5). Single nucleotide polymorphisms can alter gene express by impacting binding, cleav-age, methylating, and the genetic differentiation between individuals are due to the degradation of mRNA of gene transcription factors (6). Hence, SNPs are a resting carcinogenic marker. Information biology analyzes based on the high-throughput sequences can help us to explore biological markers of early diagnosis, pathogenesis and new therapeutic targets of tumors. If tumor-related SNPS can be detected and corrected, such precision therapy may lead to further progress in our research on tumors. TCGA database (https://portal.gdc.cancer.gov/) as the biggest cancer gene information database, includes gene express data, mRNA expression data and copy number changes, DNA methylation, SNPS and other data (7). It is of great help in a overall analysis of the express of these components in different cancer forms (8). The goal is to help research into individualized, precise treatments for colon cancer.

Methods

data treating and analysis. The raw SNP data in TCGA is not available. We have obtained the processed SNP data of COAD and the original mRNA expression data. A total of 514 specimens were collected, including 41 normal specimens and 473 cancer specimens. From the SNP data of the COAD samples, we obtained the mutated gene. The downloaded SNP data was consolidated and analyzed using the maftool and genvir packages. The data afforded by TCGA is outward and open, so approval from a indigenous ethics council is not required.

functional richness and pathway analysis of mutated genes. We used the R package "ClusterProfiler" to annotate the function of the mutated (9). Both the gene ontology (GO) and the Kyoto encyclopedia of genes and genomes (KEGG) can assess the relevant function of categories. GO and KEGG enrichment pathways were significant when p and q values were less than 0.05.

mutation and expression. We set two conditions to screen out meaningful genes. The expression difference between the mutant group and the wild group was statistically significant, and the mutant gene was associated with the prognosis of patients. To analyze CTTNBP2 / DAPK1 / DMXL2 / SPTBN2 gene expression in total, the correlation coefficient of filter conditions was 0.3, p value was 0.001. After screening the genes with the most significant expression of CTTNBP2 / DAPK1 / DMXL2 / SPTBN2, CTTNBP2 / DAPK1 / DMXL2 / SPTBN2 correlation analysis circle was drawn with the corrplot"and circlize" packages. The aim was to identify the genes similar to the core gene expression pattern, identify the most likely regulatory genes, and explore the potential mechanism of these core genes affecting prognosis.

drug sensitivity analysis. GSCALite is a cancer gene set analysis platform (10) It can integrate the cancer genomics data of 33 cancer types from TCGA, the drug response data from GDSC and CTRP, and the normal tissue data from GTEx, and it can also conduct the gene set analysis in the data analysis process. In this study, the sensitivity of CTRP to key hub genes was analyzed by GSCALite.

the relationship between pivotal genes and immune cells. The TIMER database (https://cistrome.shinyapps.io/timer/) is the use of RNA - Seq expression spectrum data detection of immune cells in tumor tissue infiltration (11). In this study, TIMER database was used to investigate the relationship between hub gene and immune cell content, and to compare the infiltration levels between tumors with different somatic cell copy number changes of hub gene (12).

gene-set difference analysis. Gene set variation analysis (GSVA) is a nonparametric and unsupervised method to evaluate the enrichment of transcriptome gene sets. GSVA determines the biological function of the sample by comprehensively scoring the gene set of interest and transforming the gene level change into the pathway level change (13). In this study, the gene sets will be downloaded from the Molecular signatures database (v7.0) and the GSVA algorithm will be used to comprehensively score each gene set to evaluate the potential changes in biological function of different samples.

Results

data processing and analysis. The SNP of COAD samples was extracted from the second generation sequencing data using the VarScan method in the TCGA database. The study identified more than 850 mutated genes in more than 30 samples, of which more than 100 were mutated in the top 10. (Figure 1) The total sample included 41 normal samples and 473 cancerous tissue samples. Further analysis of these mutated genes will provide a better understanding of their biological functions.

functional enrichment and pathway analysis of mutant genes. In order to further understand the function of mutated genes in COAD, we used R package "ClusterProfiler" to conduct functional enrichment analysis and pathway analysis of 850 mutated genes in more than 30 samples. Those mutated genes associated with COAD are enriched in a variety of pathways affecting biological processes (BPs) and cellular components (CCs) (Figure 2). SNP mutated genes are enriched in multiple tumor signaling pathways, including PI3K–Akt signaling pathway, focal adhesion, and calcium signaling pathway (figure 3). Functional analysis showed that in BPs, SNPs mutated gene was mainly concentrated in synaptic tissue and cell adhesion. In the CC group, SNPs are enriched in extracellular matrix and collagen–containing extracellular matrix (Figure 2).

mutation and expression. The screened SNPs mutated genes (CTTNBP2, DMXL2, DAPK1 and SPTBN2) were significantly correlated with patient prognosis (Figure 4). The patients with low expression of CCTNBP2 have worse prognosis than those with high expression. The patients with high expression of DAPK1、DMXL2、SPTBN2 have worse prognosis than those with low expression. In terms of gene expression, DMXL2, DAPK1 and SPTBN2 in the mutant group are higher than those in the wild group. However, the expression level of CTTNBP2 mutant sample group is significantly lower than that of the wild group (Figure 5). Using RNA-seq data, we selected the top ten genes that expressed the most significantly with the hub-genes. We identified the top ten genes most similar to the core genes, so that we could further analyze the genes that are most likely to regulate, and explore the underlying mechanisms by which these Hub-genes affect prognosis (Figure 6). According to the picture, CFTR、

SHROOM4 as well as LSM8 are positively related to CCTNBP2. TBC1D8、RNF144B、TRIB2、FAM114A1、CD109、DUSP4、EPHA4、ASPHD2、HPSE、LAG3 are positively related to DAPK1. TNRC6B、PCNX1、TET2、RIC1、ARID2 are positively related to DMXL2. TECR、ATP5MC2、RBM42、UBX1、MD5D5 have a negative correlation with DMXL2. All the ten genes in the figure are positively correlated with SPTBN2 (Figure 6 D).

drug sensitivity analysis. The expression of Hub genes affects the prognosis of colon cancer patients, so we did a drug sensitivity analysis of the Hub genes to observe which drugs affect their expression. The picture shows that SNS-032, Paclitaxel, Nsc23766 and BRD-K66453893 drugs can cause the high expression of CTTNBP2. Class MLN2480 drugs can reduce the expression of the DMXL2. The expression of SPTBN2 can be inhibited by austocystin D, afatinib, belinostat, lapatinib, ibrutinib (Figure 7).

The tumor pathway map shows that CTTNBP2 gene is mainly related to apotposis and cell cycle and is involved in the inhibitory effect. The DNPK1 and DMXL2 genes are primarily involved in activating the RTK and TSC/mTOR pathways. SPTBN2 gene activates apotposis pathway (Figure 8).

the relationship between pivotal genes and immune cells. We used the timer database to analyze the correlation between the expression of core genes and several important immune cells in order to understand whether core genes can influence the prognosis of patients in the way of immune infiltration. We observed that the DNPK1and DMXL2 genes were significantly associated with several important immune cells.

gene-set difference analysis. In order to further study the signaling pathway of core genes in tumors, we conducted GSVA analysis of core genes in tumor samples and non-malignant samples, respectively. Among them, gene DMXL2 and gene DAPK1 are enriched in the Uv respose up, DNA repair and myc target V2 pathways, indicating that they may be of important significance in tumorigenesis and development.

Discussion

COAD is one of the most leading cause of human death in the world. Early detection of SNP mutations was conducted to find meaningful biological markers to predict the occurrence and development of tumors. (4) (5) (6) After functional enrichment and pathway analysis, researchers began to further study the molecular mechanisms of these mutated genes.

In our study, we screened out SNP mutated genes through bioinformatics analysis of coad-related genes based on TCGA data. Functional enrichment and pathway analysis were performed to further investigate the direct involvement of mutated genes in relevant molecular mechanisms. Outside the cell, genes accumulate in the matrix and collagen–containing extracellular matrix. The genes basically coordinate the transmission of signals, such as the formation of new synapses, cell adhesion, and actin binding. According to the results of pathway analysis, these mutated genes are related to the pi3k-akt signaling pathway, calcium signaling passage and focal adhesion, and that these pathways were associated with tumor evolution. The enrichment function and the study of the pathway reveal the molecular mechanism of SNP mutations in disease progression. The express levels about the four hub genes (CTTNBP2,

DAPK1, DMXL2, SPTBN2) were significantly different from those of the wild type. The expression of these genes was also significantly correlated with patient prognosis. Analysis of coexpression, drug sensitivity and multiple levels of immune infiltration of these core genes can better help us understand how they affect the prognosis of patients.

CTTNBP2 can influence the shape and number of dendritic spines (14). CTTNBP2 also plays a role in other tissues, including the binding of the rad21 - cohesive protein complex (15). For prostate cancer, specifically, CTTNBP2 is located in a gene block on chromosome 7, a gene with differential methylation in the prostate cancer cell line (16). Our study indicated that the low expression of CTTNBP2 gene meaned that patients with COAD had a worse prognosis. The gene set associated with the co-expression of CTTNBP2 was significantly related to the development of tumor. For example, the overexpression of JADE3, in vivo and in vitro, the stem-like properties of colon cancer cells increased and inhibition decreased. JADE3 must be expressed, so that the tumorigenic characteristics of cancer cells in the body can reduce the damage to the human body. The promoters of the JADE3 interaction (colon cancer stem cell markers) and LGR5 promoted acetyltransferase and histone acetylation of p300 in terms of activated transcription, so significantly induced Wnt/jp-catenin signaling to some extent (17). For another example, NORAD (Non-Coding RNA Activated By DNA Damage) is an RNA Gene, and is affiliated with the lncRNA class. The diseases associated with NORAD include pancreatic cancer and bladder cancer. NORAD is one of the independent prognostic factors for each patient with PDAC, which can promote the metastasis and invasion of PDAC in vivo and in vitro. (18). Particularly, NORAD may act as ceRNA and control the expression of RhoA, a small GTP binding protein, by competing for hsa-mir-125a-3p, thereby promoting the generation of EMT. (18) In our study, CTTBNBP2 is involved in multiple tumor pathways. Its expression inhibits cell apoptosis and cell cycle. This suggests that its low expression predicts a worse prognosis.

DAPK1 (Death Associated Protein Kinase 1) is a Protein encoding gene. Diseases such as pancreatic ductal adenocarcinoma and cervical squamous cell carcinoma are associated with DAPK1 (19). Its related pathways are Cdk-mediated phosphorylation and removal of Cdc6 and autophagy-animal . Recent studies have linked the DAPK1 to breastand colon cancer (20) (21) RNF144B related to the expression of DAPK1, Apoptosis was induced by a p53/ t53-dependent but caspase independent mechanism. Overexpression of p53RFP promoted apoptosis of wild-type HCT116 cells without p53 deletion (22) Heparinase (HPSE) is evident in a variety of malignancies: for example, the stomach, ovary, kidney, head and neck, colon, pancreas, bladder, brain, prostate, breast and liver cancer, ewing's sarcoma, multiple myeloma, and b-lymphoma (23) (24) (25) (26)

The overexpression of HPSE gene can enhance the function of vascularization. (27) Entering a vicious circle, high HPSE levels produced by cancer cells promote angiogenesis, further promoting tumor growth. The expression of DAPK1 is associated with a variety of important tumor pathways, including TSC / mTOR, RTK pathways. This is consistent with our research (Figure 8).

DMXL2 is encoded as a protein that has 12 WD domains and thus can participate in many functions, including those involved in semaphore transduction ways. Relevant studies have shown that DMXL. Aberrant Notch signaling has been linked to breast cancer formation and progression (28) (29). Normally, mTOR is one of the significant regulators of cell proliferation and division. But in tumor cells, the abnormally activated mTOR signals encouragement for tumor cells to grow, metastasize, and invade new healthy tissue (30). The overactivation of its pathway can satisfy the rapidly growing tumor cells to get more nutrients (31). Both DMXL2 and DAPK1 genes are involved in the activation of this pathway.

SPTBN2 (Spectrin Beta) is a protein-encoded genotype. SPTBN2 stabilizes glutamate transporters on the surface of cell membranes, and EAAT4 regulates the glutamate signaling way. High expression of SPTBN2 means that patients with colon cancer have a worse prognosis. Relevant reports have shown that gene PLXNA1, which is significantly co-expressed with gene SPTBN2, is involved in the MAPK pathway. Silencing the expression of gene A can enhance cell apoptosis in esophageal squamous cell carcinoma, block cell cycle, inhibit cell growth, removal, and invade (32).Immunoin filtration analysis showed that the expressions of DAPK1 and DMXL2 DAPK1 and DMXL2 are likely to play an significant role in the research of immune cell infiltration and is a biomarker for the prognosis of patients with colon cancer.

Conclusion

Bioinformatics analysis showed that there were four core genes in the SNPS mutation data, and the expression differences between the wild type and the mutant were significant, and the prognosis of patients was significantly different. Further analysis revealed that the core genes and the genes associated with their expression, there are many different ways in which cancer can develop. Besides, the expressions of DAPK1 and DMXL2 are associated with a variety of important immune cell infiltrations. All these findings strongly suggested that the detection of these mutated genes and their expression levels could be used to check the patient's prognosis. Let's go a little bit further an analyze the drug sensitivity of the core genes, providing a theoretical basis for clinical treatment. These discoveries require extensive clinical studies to demonstrate its sensibility and accuracy. The research result is an significant theoretical foundation for bioinformatics researchfor further study of colon cancer patients.

Abbreviations

SNP:single nucleotide polymorphisms

GO: gene ontology

KEGG:Kyoto encyclopedia of genes and genomes

GSVA:Gene set variation analysis

GSCALite:A web server for Gene Set Cancer Analysis

TIMER:Tumor immune Estimation Resource

Declarations

Ethics and Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the author on reasonable request.

Competing Interest

The author reports no conflicts of interest in this work.

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Not applicable.

Authors' contributions

J.L., X,L ,HJ L contributed to study design; J.L. Y,L and C,L. wrote the manuscript;X,Y,C and D,T,S revised the manuscript; X,L HJ,Land H,B,D. analyzed the data.

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References

1. Christina Fitzmaurice, Tomi F Akinyemiju, Faris Hasan Al Lami, Tahiya Alam, Reza Alizadeh-Navaei, Christine Allen, Ubai Alsharif, Nelson Alvis-Guzman, Erfan Amini, Benjamin O AndersonGlobal et al: Regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability- adjusted life-years for 29 cancer groups, 1990 to 2016: A systematic analysis for the global burden of disease study. JAMA Oncol 4: 1553-1568, 2018

2. Brenner, Hermann Kloor, Matthias Pox and Christian Peter: Colorectal cancer. Lancet 383: 1490-1502, 2014.
3. Ravi Sachidanandam, David Weissman, Steven Schmidt, Jerzy M Kakol, Lincoln Stein, Gabor T Marth, Steve Sherry, James C Mullikin, Beverley Mortimore, David Willey et al: A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 409: 928-933, 2001.
4. Oksana Moshynska, Igor Moshynskyy, Vikram Misra and Anurag Saxena: G125A single-nucleo-tide polymorphism in the human BAX promoter affects gene expression. Oncogene 24: 2042-2049, 2005.
5. Joanna Somers, Lindsay A Wilson, John-Paul Kilday, Emilie Horvilleur, Ian G Cannell, Tuija Poyry, Laura C Cobbold, Alexander Kondrashov, JohnR P Knight and Stephanie Puget: A common polymorphism in the 5' UTR of ERCC5 creates an upstream ORF that confers resistance to platinum-based chemotherapy. Genes Dev 29: 1981-1896, 2015.
6. Anjali Shukla, Jude Alsarraj and Kent Hunter: Understanding susceptibility to breast cancer metastasis: the genetic approach. Breast Cancer Manag 3: 165-172, 2014.
7. Katarzyna Tomczak, Patrycja Czerwińska, Maciej Wiznerowicz. The cancer genome atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 19: 68-77, 2015.
8. Ethan Cerami, Jianjiong Gao, Ugur Dogrusoz, Benjamin Gross, Selcuk Onur Sumer, Bulent Arman Aksoy, Anders Jacobsen, Caitlin J Byrne, Michael Heuer, Erik Larsson et al: The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discover 2: 401-404, 2012.
9. Guangchuang Yu, Li Gen Wang, Yanyan Han and Qingyu He: ClusterProfiler: an r package for comparing biological themes among gene clusters. OMICS 16: 284–287, 2012.
10. Chun Jie Liu, Fei Fei Hu, Meng Xuan Xia, Leng Han, Qiong Zhang and Anyuan Guo: GSCALite: a web server for gene set cancer analysis, Bioinformatics 34: 3771–3772, 2018.
11. Taiwen Li, Jingyu Fan, Binbin Wang, Nicole Traugh, Qianming Chen, Jun S Liu, Bo Li, X Shirley Liu: TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res 77: 108–110, 2017.
12. Bo Li, Eric Severson, Jeanchristophe Pignon, Haoquan Zhao, Taiwen Li, Jesse Novak, Peng Jiang, Hui Shen, Jon C Aster, Scott J Rodig et al: Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome Biol 17: 174, 2016.
13. Aravind Subramanian, Pablo Tamayo, Vamsi K Mootha, Sayan Mukherjee, Benjamin L Ebert, Michael A Gillette, Amanda G Paulovich, Scott L Pomeroy, Todd R Golub, Eric S Lander et al: Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102: 15545–15550, 2005.
14. Yikai Chen and Yiping Hsueh: Cortactin-binding protein 2 modulates the mobility of cortactin and regulates dendritic spine formation and maintenance. The Journal of Neuroscience 32: 1043–1055, 2012.

15. Anil K Panigrahi, Nenggang Zhang, Subhendu K Otta and Debananda Pati: A cohesin-RAD21 interactome. Biochem J 442: 661–670, 2012.
16. Marcel W Coolen, Clare Stirzaker, Jenny Z Song, Aaron L Statham, Zena Kassir, Carlos S Moreno, Andrew N Young, Vijay Varma, Terence P Speed, Mark J Cowley et al: Consolidation of the cancer genome into domains of repressive chromatin by long-range epigenetic silencing (LRES) reduces transcriptional plasticity. Nat Cell Biol 12: 235–246, 2010.
17. Yunting Jian, Meng Wang, Yan Zhang, Ruizhang Ou, Ziyuan Zhu, Yangying Ou, Xiangfu Chen, Xin Liang, Yanqing Ding, Libing Song et al: Jade family PHD finger 3 (JADE3) increases cancer stem cell-like properties and tumorigenicity in colon cancer. Cancer Letters 428: 1–11, 2018.
18. Hongzhe Li, Xinjing Wang, Chenlei Wen, Zhen Huo, Weishen Wang, Qian Zhan, Dongfeng Cheng, Hao Chen, Xiaxing Deng, Chenghong Peng et al: Long noncoding RNA NORAD, a novel competing endogenous RNA, enhances the hypoxia-induced epithelial-mesenchymal transition to promote metastasis in pancreatic cancer. Mol Cancer 16: 169, 2017.
19. Qinghua Feng, Stephen E Hawes, Joshua E Stern, Amadou Dem, Papa Salif Sow, B Dembele, Papa Toure, Pavel Sova, Peter W Laird, Nancy B Kiviat: Promoter hypermethylation of tumor suppressor genes in urine from patients with cervical neoplasia. Canc Epidemiol Biomarkers Prev 16: 1178–1184, 2007.
20. Jing Zhao, Dekuang Zhao, Graham M Poage, Abhijit Mazumdar, Yun Zhang, Jamal Hill, Zachary C Hartman, Michelle I Savage, Gordon B Mills, Powel H Brown: Death-associated protein kinase 1 promotes growth of p53-mutant cancers. J Clin Invest 125: 2707–2720, 2015.
21. Sara Steinmann, Philipp Kunze, Chuanpit Hampel, Markus Eckstein, Jesper B Bramsen, Julienne K Muenzner, Birgitta Carle, Benardina Ndreshkjana, Stephan Kemenes, Pierluigi Gasparini et al: DAPK1 loss triggers tumor invasion in colorectal tumor cells. Cell Death Dis 10: 895, 2019.
22. Jun Huang, Liangguo Xu, Ting Liu, Zhonghe Zhai and Hongbing Shu: The p53-inducible E3 ubiquitin ligase p53RFP induces p53-dependent apoptosis. FEBS Lett 580: 940–947, 2006.
23. Ilan N, Elkin M and Vlodavsky I: Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. Int J Biochem Cell Biol 38: 2018–2039, 2006.
24. Israel Vlodavsky, Phillip Beckhove, Immanuel Lerner, Claudio Pisano, Amichai Meirovitz, Neta Ilan and Michael Elkin: Significance of heparanase in cancer and inflammation. Cancer Microenviron 5:115–132, 2012.
25. Vreys V and David G: Mammalian heparanase: What is the message? J Cell Mol Med 11: 427–452, 2007.
26. Maria Francesca Secchi, Valentina Masola, Gianluigi Zaza, Antonio Lupo, Giovanni Gambaro and Maurizio Onisto: Recent data concerning heparanase: Focus on fibrosis, inflammation and cancer. Biomol Concepts 6: 415–421, 2015.
27. Eyal Zcharia, Shula Metzger, Tova Chajekshaul, Helena Aingorn, Michael Elkin, Yael Friedmann, Talia Weinstein, Jinping Li, Ulf Lindahl, Israel Vlodavsky: Transgenic expression of mammalian

heparanase uncovers physiological functions of heparan sulfate in tissue morphogenesis, vascularization, and feeding behavior. FASEB J 18: 252–263, 2004.

28. Nilay Sethi, Yan Yan, Debra Quek, Trudi Schupbach and Yibin Kang: Rabconnectin-3 is a functional regulator of mammalian notch signaling. J Biol Chem 285: 34757–34764, 2010.
29. Victoria Bolos, Emilia Mira, Beatriz Martinezpoveda, Guillermo Luxan, Marta Canamero, Carlos Martineza, Santos Manes and Jose Luis De La Pompa: Notch activation stimulates migration of breast cancer cells and promotes tumor growth. Breast Cancer Res 15: 1-15, 2013.
30. Andrew C Hsieh, Yi Liu, Merritt P Edlind, Nicholas T Ingolia, Matthew R Janes, Annie Sher, Evan Y Shi, Craig R Stumpf, Carly Christensen, Michael Bonham, et al: The translational landscape of mTOR signalling steers cancer initiation and metastasis. Nature 485: 55–61, 2012.
31. Yakir Guri, Marco Colombi, Eva Dazert, Sravanth K Hindupur, Jason Roszik, Suzette Moes, Paul Jenoe, Markus H Heim, Isabelle Riezman, Howard Riezman et al: mTORC2 promotes tumorigenesis via lipid synthesis. Cancer Cell 32: 807, 2017.
32. Weiwei Wang, Zhihua Zhao, Li Wang, Pan Li, Kuisheng Chen, Jianying Zhang, Wencai Li, Guozhong Jiang and Xiangnan Li: MicroRNA-134 prevents the progression of esophageal squamous cell carcinoma via the PLXNA1-mediated MAPK signalling pathway. EBioMedicine 46: 66–78, 2019.

Figures

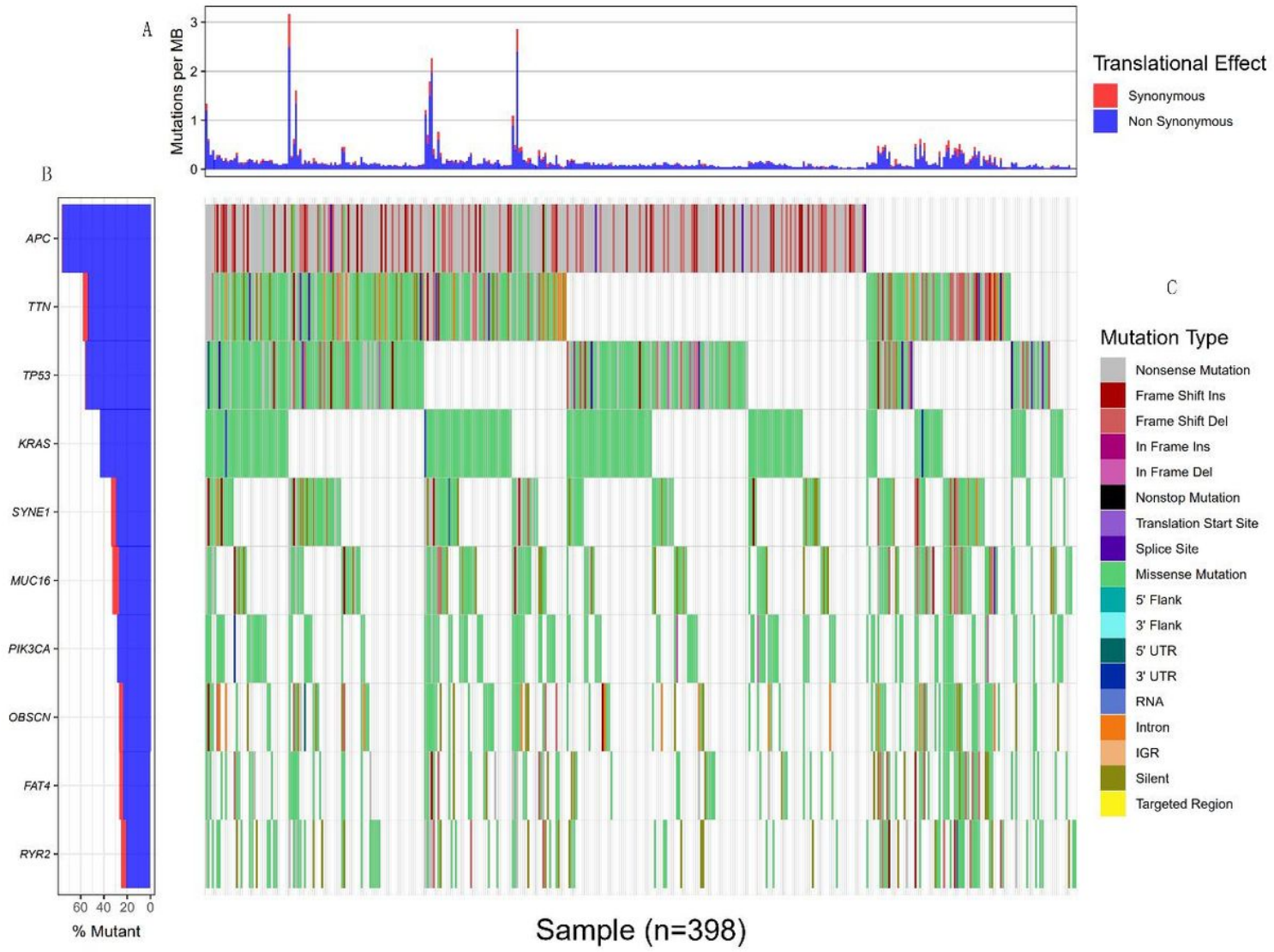

Figure 1

A waterfall map of 10 genes that mutated in more than 100 samples. (A) tanslational effect (B) mutated gene and (C) mutation type.

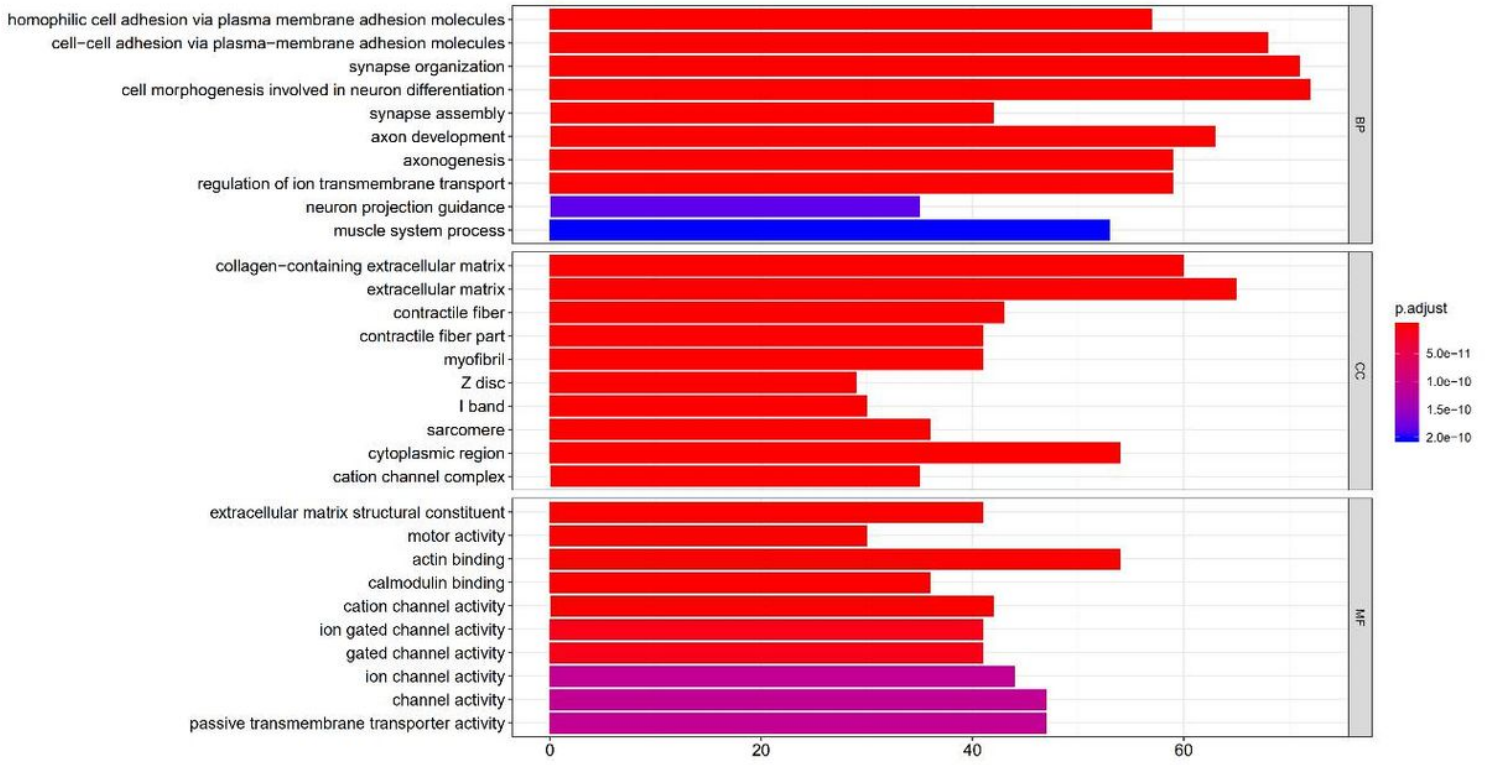

Figure 2

Top 10 terms were selected according to count and P value <0.05. Count: the number of enriched genes in each term.

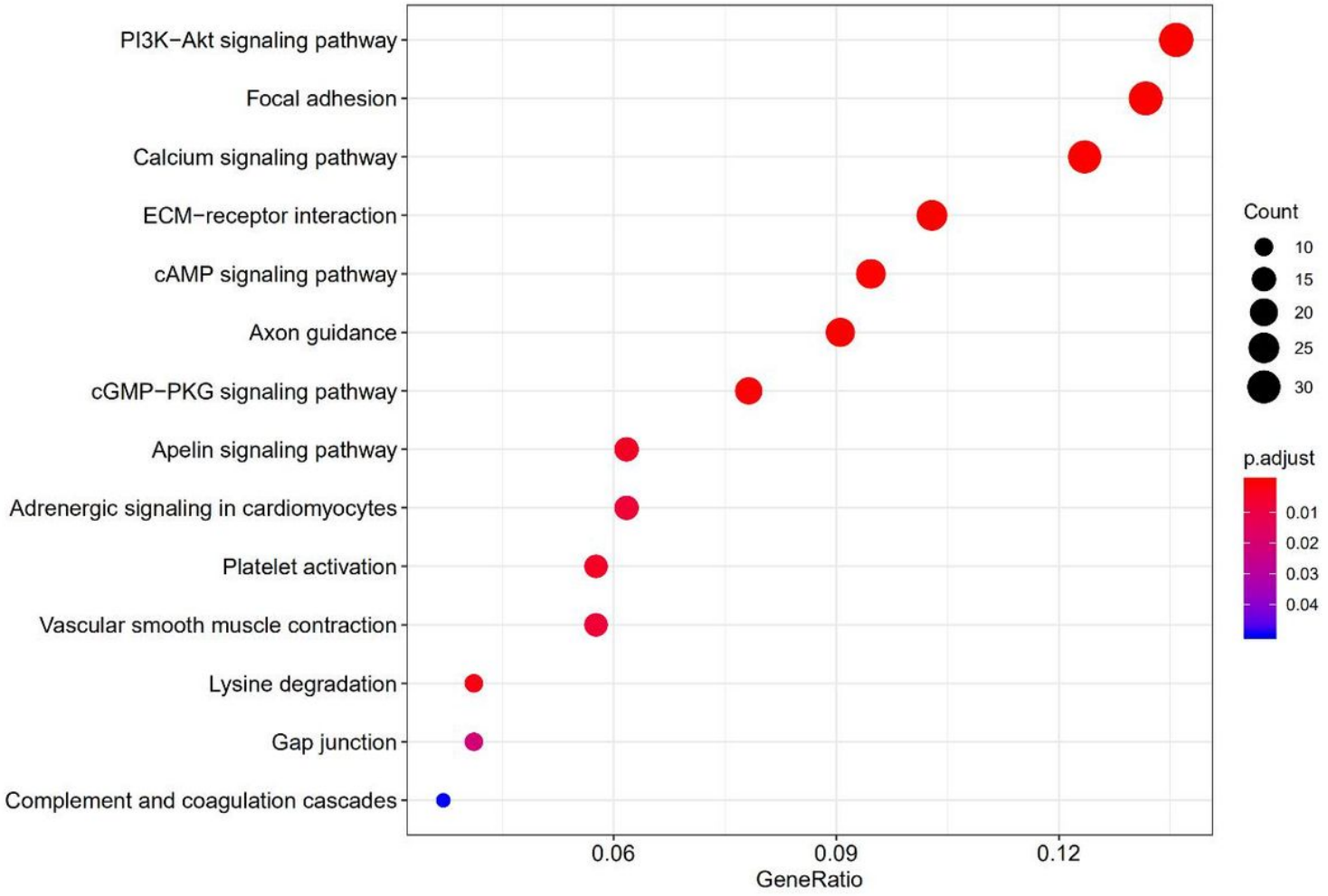

Figure 3

Pathways enrichment map of 850 mutant genes. The top 20 terms with the lowest p value were selected. Count: the number of enriched genes in each term.

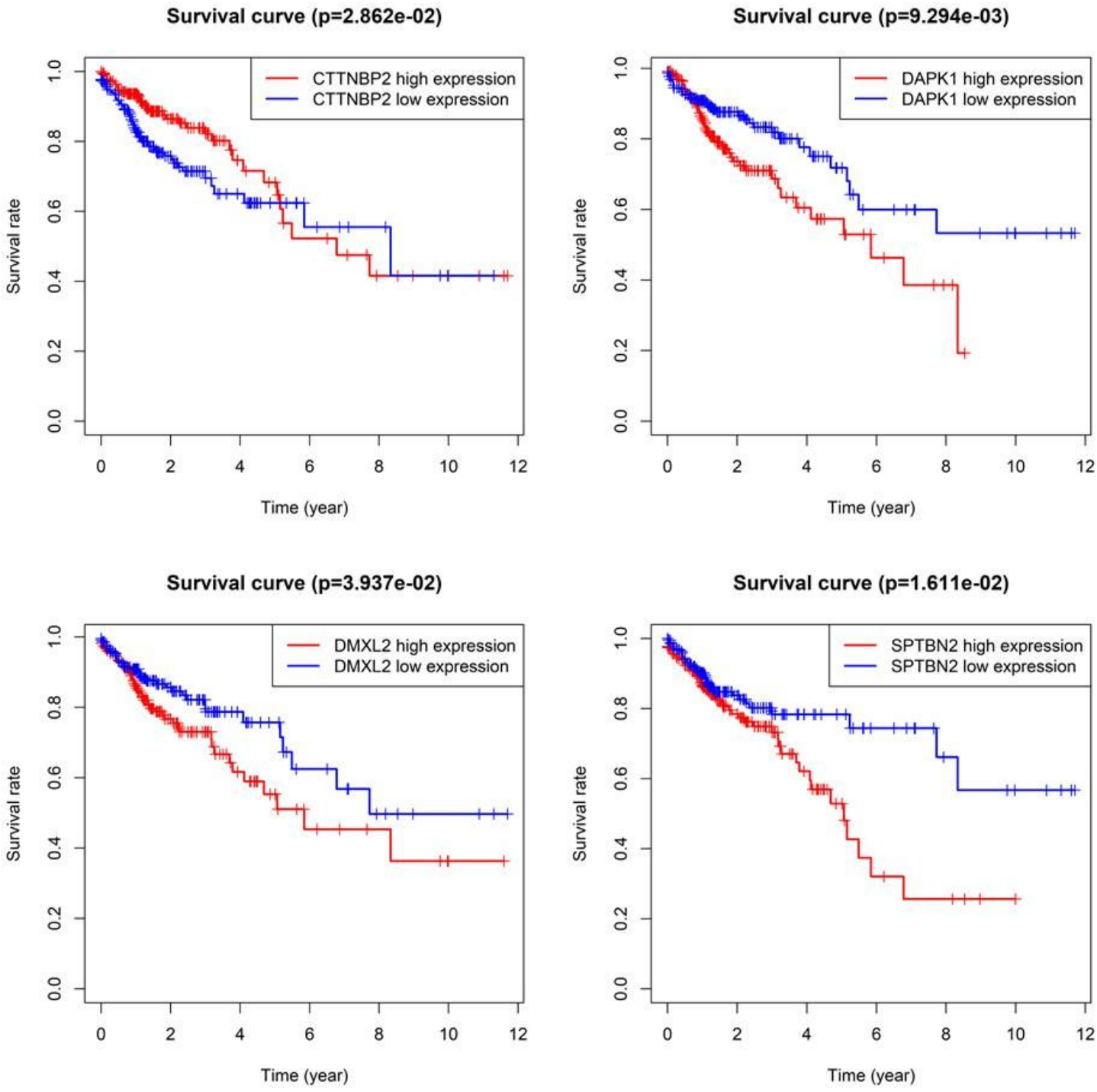

Figure 4

Kaplan-Meier survival curves of the mutant genes.

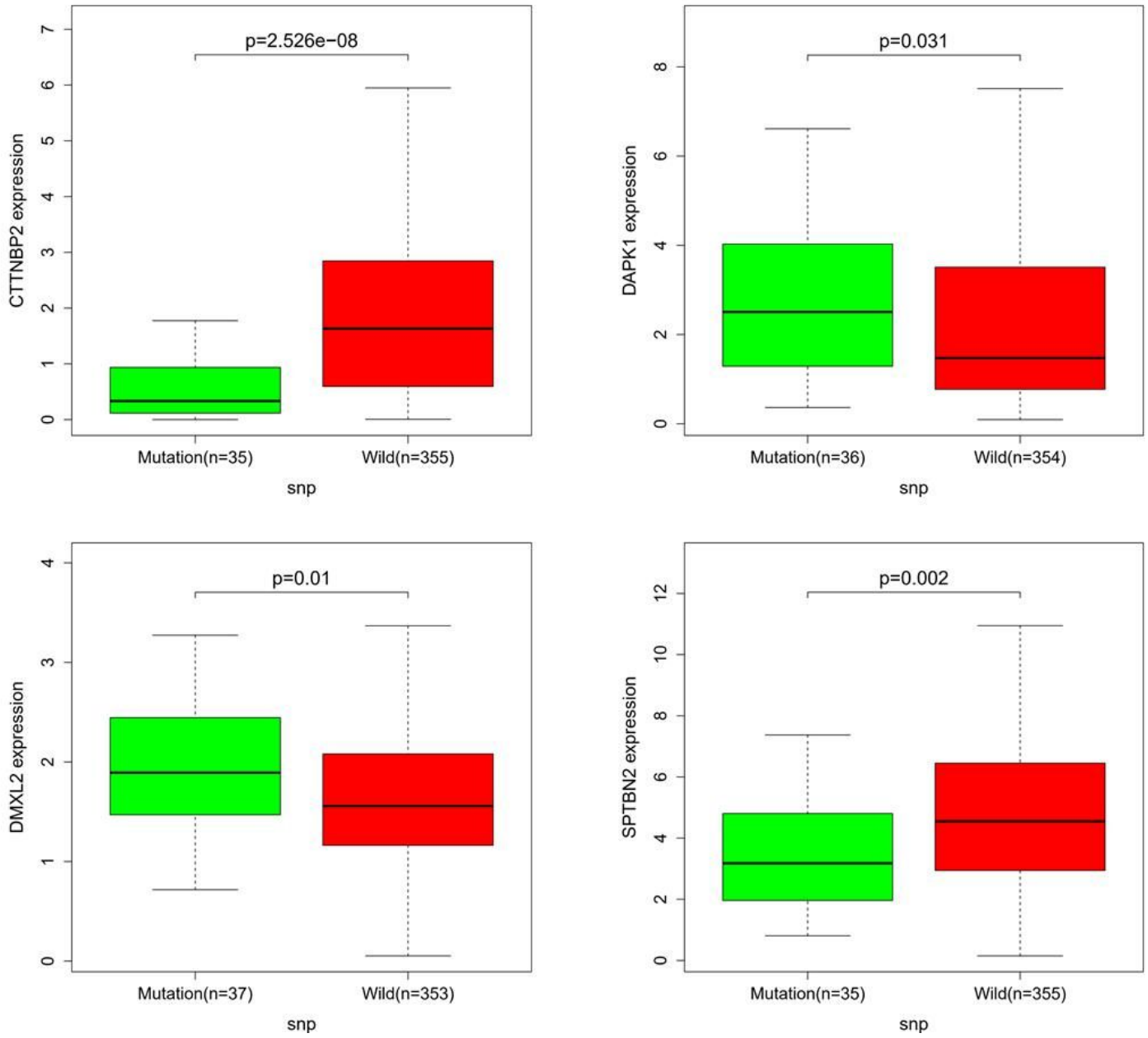

Figure 5

The relationship between mutation and expression about four genes.

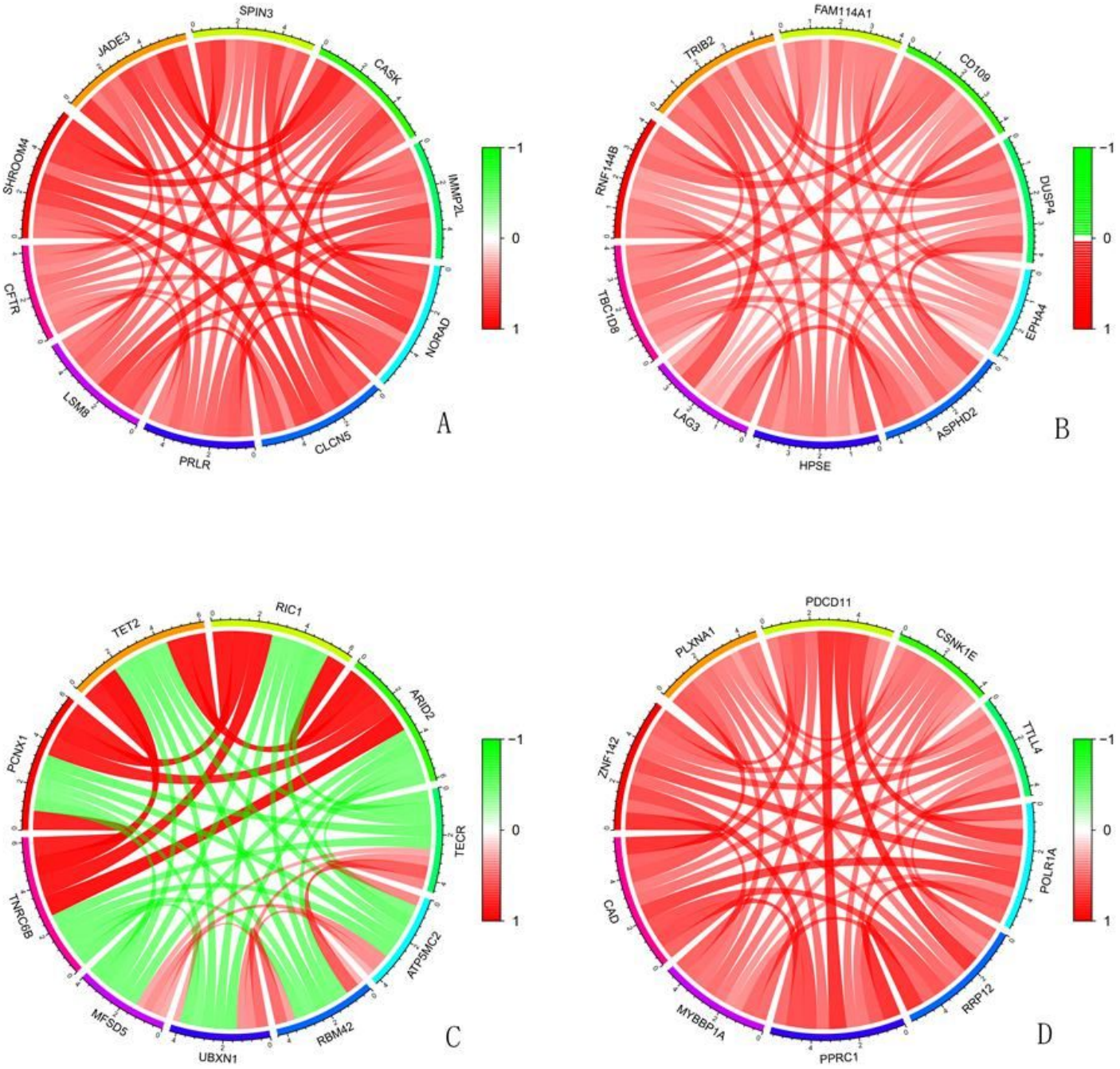

Figure 6

The top 10 genes were significantly correlated with the expression of four Hub-genes: (A) Ten genes that were clearly associated with CTTNBP2 (B) Ten genes that were clearly associated with DAPK1 (C) The top five genes with the most negative correlation to gene DMXL1 and the top five genes with the most positive correlation to gene DAPK1 (D) Ten genes that were clearly associated with SPTBN2.

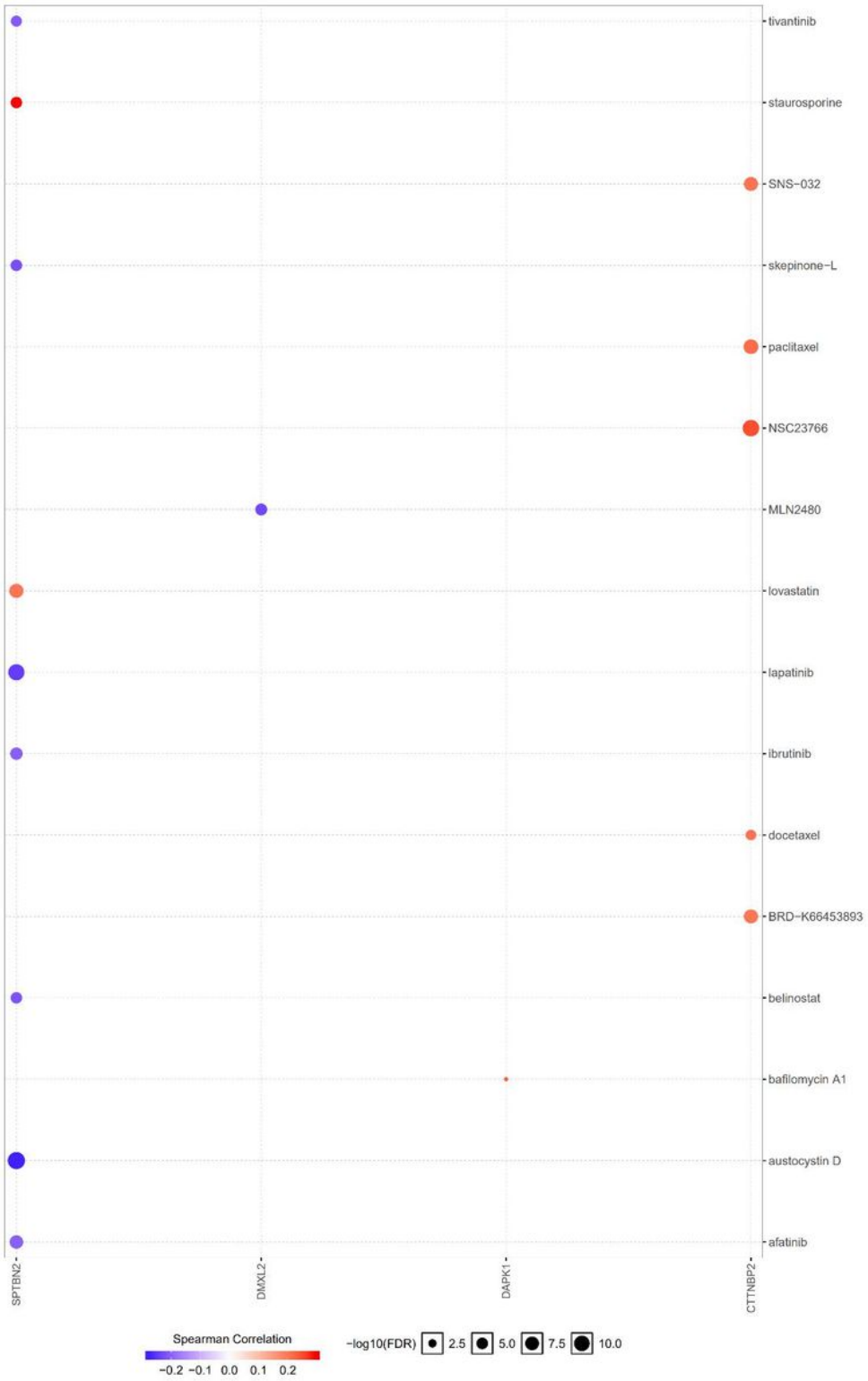

Figure 7

Drug sensitivity analysis of Hub-genes.

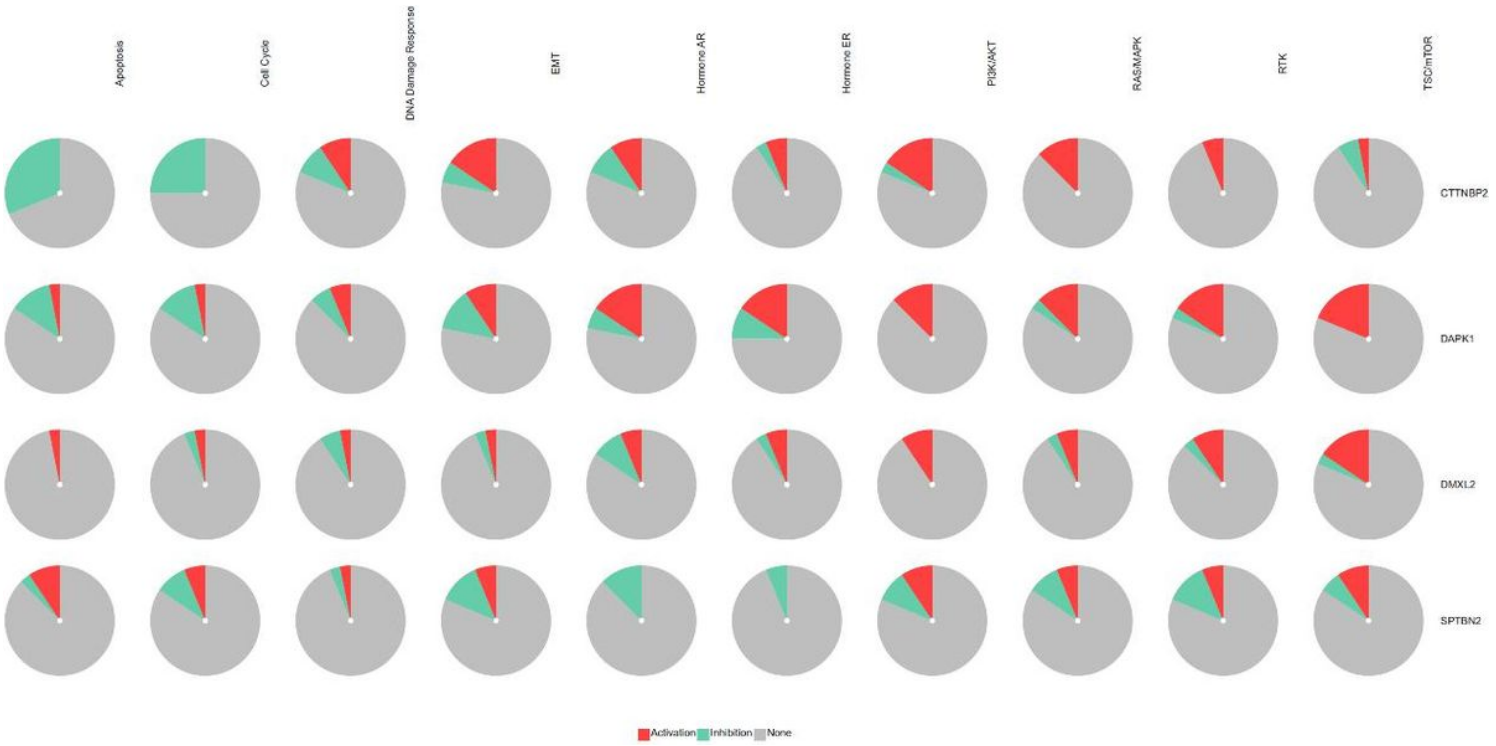

Figure 8

Tumor pathway activity of Hub genes.

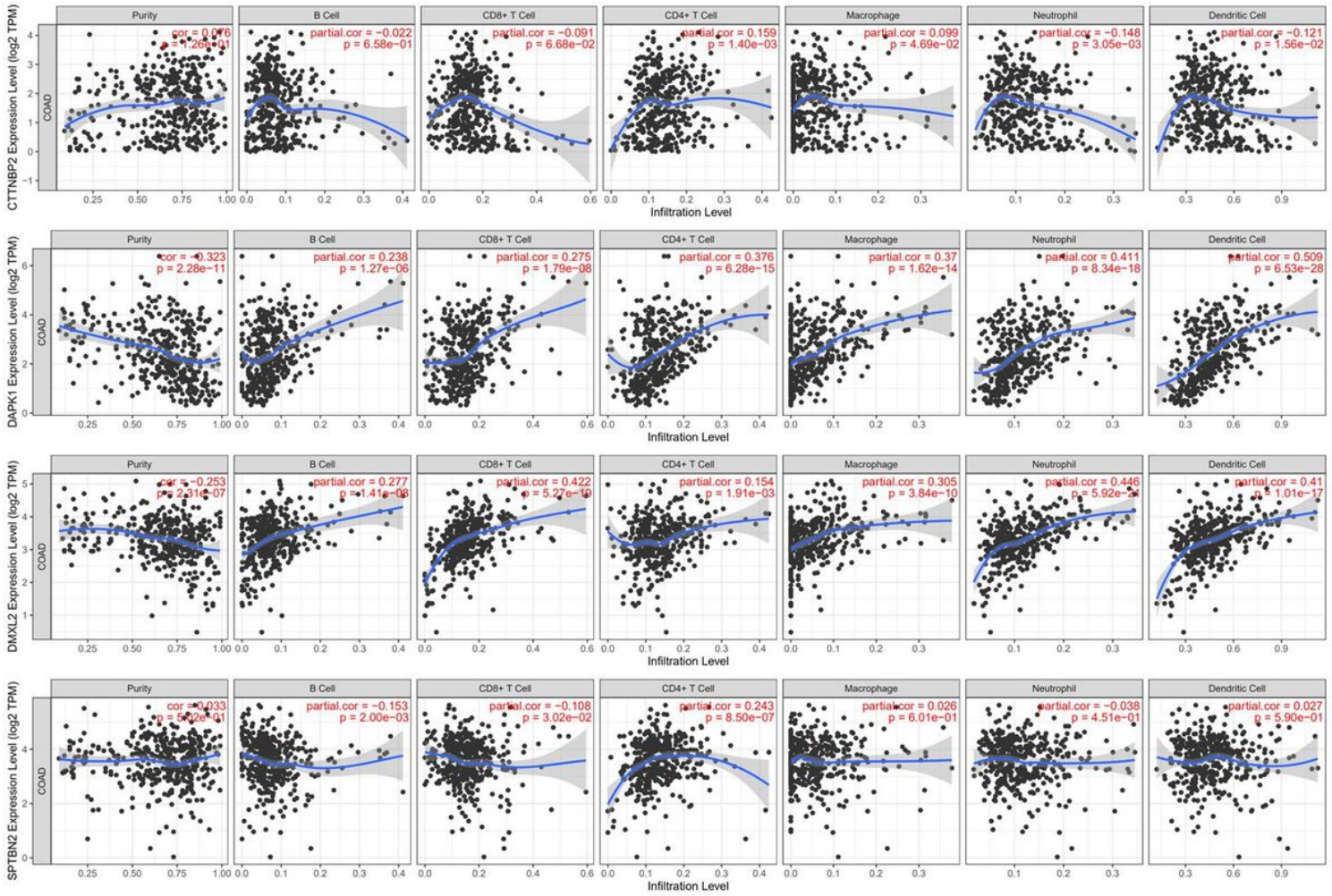

Figure 9

Integrative analysis between hub identified immune signature with tumor-only immune cells.

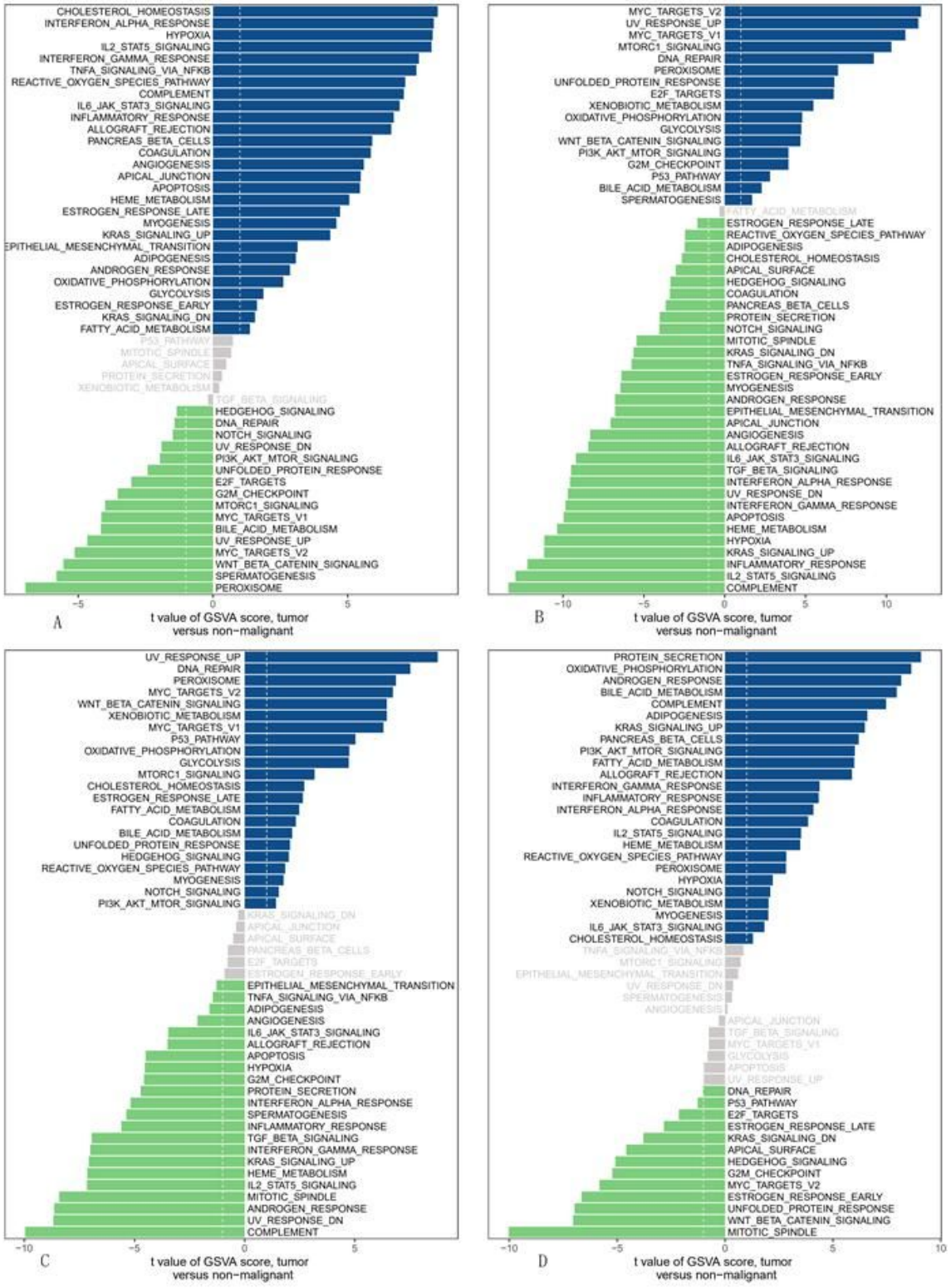

Figure 10

GSVA analysis of Hub-genes: (A) CTTNBP2 (B) DAPK1 (C) DMXL2 (D) SPTBN2.