Identification of critical pathways and hub genes in *TP53* mutation prostate cancer by bioinformatics analysis

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Aim: The *TP53* mutant is one of the most common mutant genes in prostate cancer. **Materials & methods:** The RNA-seq data of prostate cancer was downloaded from TCGA database. Gene set and enrichment analyses were done by online tools. **Results:** *TP53* mutation was found in 18% prostate cancer patients. Enrichment analysis indicated that differentially expressed genes were enriched in GPCR signaling pathways, cell growth and metabolism. The top ten hub genes were identified. Further analysis showed increased risk of recurrence, lower *TP53* mRNA level and higher Gleason scores in patients with *TP53* mutation. **Conclusion:** Our results suggest that multiple genes and pathways may play key roles in *TP53* mutant prostate cancer, providing candidate targets and strategies for individualized treatment.

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Keywords: bioinformatics analysis • prostate cancer • RNA sequencing • TCGA • *TP53* mutation

In developed countries, prostate cancer is still the leading cause of cancer-related mortality in men [1]. Although many studies focus on the genes, signaling pathways and cellular processes that contribute to cell invasion, the underline mechanism is still not fully understood. Recently, as prostate cancer is hereditary, more and more research contributes to dig out substantial heterogeneity among primary prostate cancers [2]. As a result, many online databases offer the spectrum of molecular abnormalities and its variable clinical course, such as The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO). Among all reported mutant genes, *TP53* is one of the most frequently mutated genes in primary prostate cancers [3].

TP53, which is encoded by the human gene *TP53*, is a tumor-suppressor protein in many tumor types. *TP53* protein has been shown to play important roles in dealing with various cellular stresses to regulate target gene expression, which induces transcriptional activation, DNA repair, apoptosis, senescence, cell cycle arrest or changes in metabolism [4]. Mutations in *TP53* have been found in multiple human cancers, including prostate cancer. Most of the observed mutation types in *TP53* are single-nucleotide missense variants, while missense mutations are the majority types of cancer-related *TP53* mutation, which result in inactivating mutation, deleterious gain of function or loss of gene function. Otherwise, *TP53* mutation might influence disease program and cancer progression, thus affects disease prognosis and therapeutic strategy [5]. Previous study on pancreatic cancer suggests that mutant *TP53* might promote the metastatic potential and accelerate proliferation of metastatic tumor cell [6]. Mutant *TP53* may result in the activation of multiple cancer-related biologic mechanisms, which contributes to disease progression [7].

As *TP53* mutation is frequently detected in prostate cancer patients, with a mutation rate in the range of 10–20% in primary tumors, we wonder whether clustering analysis of data from molecular profiling of tumors would define *TP53* mutant tumors as a subgroup [8]. However, the changes in biological processes and clinical attributes *TP53* mutation cause in prostate cancer still remain unclear. In this study, we analyzed an RNA sequencing (RNA-Seq) dataset of prostate cancer to identify the key pathways and genes associated with *TP53* mutation using bioinformatics analysis approaches, expecting to uncovered the potential role of *TP53* mutation in serving as a prediction factor for prognosis and individualized treatment options.

Materials & methods

RNA-Seq data

An RNA-Seq dataset of prostate cancer with corresponding clinical profiles was downloaded from TCGA database (<https://portal.gdc.cancer.gov/>). Related information was obtained from the online dataset cBioPortal for Cancer Genomics website (www.cbioportal.org/index.do) [9].

Gene set enrichment analysis

We analyzed gene set enrichment analysis (GSEA) v3.0 (<http://software.broadinstitute.org/gsea/downloads.jsp>) to dig out the differences in gene mRNA expression levels between both groups. The results indicated the effect of *TP53* mutation on various biological function gene sets in prostate cancer patients. The number of permutations was set at 10. In analysis, we used the following value as cut-off: nominal p-value cut-off of 0.05; false discovery rate (FDR q-val) <0.25.

Identification of differentially expressed genes

EdgeR, an R package for examining differential expression of RNA-Seq count data as previously described [10,11]. Differentially expressed genes (DEGs) were identified with the following criterion: fold change (FC) ≥ 2 or ≤ 0.5 ; the p-value and FDR <0.05.

Functional annotation & pathway enrichment analysis

We used the database for annotation, visualization and integrated discovery (DAVID) website to undergo GO analysis and KEGG analysis [12], while specifying a p-value <0.05 for statistical significance.

Integration of protein–protein interaction network & module analysis

We used Search Tool for the Retrieval of Interacting Genes (STRING) online database to do assessment and integration of protein–protein interaction (PPI) [13]. We uploaded the DEGs to STRING online website and then evaluated the interactive relationships. The cut-off is combined score more than 0.4. We next imported the analyzed PPI network into Cytoscape to complete module screening by Molecular Complex Detection (MCODE; cut-off: scores >3 and nodes >4) [14].

Statistical analysis

The Student t-test was used to compare the *TP53* mRNA expression level and Gleason score between *TP53* mutation and wild-type prostate cancer tissue. The Kaplan–Meier method was used for analyzing the biochemical recurrence percentage between different *TP53* status groups by GraphPad. FDR in edgeR and GSEA were adjusted for multiple testing with the Benjamini–Hochberg procedure, respectively [15,16]. We used $p < 0.05$ as the level for statistically significant. All the statistical analyses were conducted with GraphPad and R 3.3.0.

Results

Data information

The information of 499 prostate cancer patients with complete follow-up profiles and corresponding cancer tissue RNA-Seq dataset were downloaded from TCGA database. There were 87 prostate cancer patients (18%) with *TP53* mutation and the rest were *TP53* wild-type (Figure 1A). Mutation types included truncating, deep deletion and missense mutations spanning over entire gene (Figure 1B). These data were obtained directly from the cBioPortal for Cancer Genomics website.

Clinical impact of *TP53* mutation on prostate cancer progression

We next investigated the influence of *TP53* mutation on prostate cancer progression. We first determined the *TP53* mRNA expression level in both groups. Results showed that *TP53* was downregulated in *TP53* mutation prostate cancer patients' tumor tissue (Figure 2A). Furthermore, we found that *TP53* mutation significantly correlate with high Gleason score (Figure 2B) and prostate cancer recurrence (Figure 2C), but not the tumor stage (Table 1).

Results of gene set enrichment analysis

To investigate the effect of *TP53* mutation on the development of prostate cancer, we analyzed the effects of *TP53* mutation on various gene sets by the GSEA. As is shown in Figure 3, the GSEA results indicate that biological

Figure 1. Mutation frequency and mutation types of *TP53* in prostate cancer. (A) Mutation frequency of *TP53* in prostate cancer. **(B)** Mutation types of *TP53* in prostate cancer reproduced from The Cancer Genome Atlas database.

Table 1. Clinical characteristics of prostate cancer patients and <i>TP53</i> mutation.			
Tumor stage	<i>TP53</i>		p-value
	Wild-type (306)	Mutation (40)	
<T2b	221	23	0.0653
>T2b	85	17	

p < 0.05 was considered statistically significant. χ^2 test was used.

processes including IFN- α response, angiogenesis, mitotic spindle, complement, G2M checkpoint, spermatogenesis, coagulation, IFN- γ response, apoptosis and oxidative phosphorylation were significantly enriched. This suggests that *TP53* mutation may affect disease progression and prognosis in prostate cancer patients by influencing mitosis, DNA repair and metabolism. The GO enrichment analysis of molecular function was significantly enriched in *E2F* targets. What is more, KEGG pathways analysis indicated that the *TP53* mutation group was associated with the *mTORC1* signaling pathway, *PI3K-AKT-mTOR* signaling pathway, *Il-2 stat5* signaling, Hedgehog signaling pathway and *TGF- β* pathway (Figure 3).

Identification of DEGs

To identify the DEGs, we analyzed the RNA-Seq datasets of 87 *TP53* mutation-bearing and other wild-type prostate cancer patients. Based on the *in silico* analysis, using p < 0.05 and fold control (FC) ≥ 2.0 criteria, a total of 950 genes were identified as DEGs, of which, 185 were upregulated and 765 were downregulated. The volcano plot of the DEGs is shown in Figure 4, while the DEGs expression heat map of top 100 genes is shown in Supplementary Figure 1.

GO & KEGG analyses of DEGs

In order to analyze the DEGs at the functional level, we submitted all the 950 DEGs online for further analyses with DAVID, respectively. The GO analysis of DEGs (Figure 5A) suggested significant enrichment in muscle filament sliding, detection of chemical stimulus involved in sensory perception of smell, GPCR signaling pathway, cardiac muscle contraction, muscle contraction, sensory perception of smell, sarcomere organization, negative regulation of growth, myofibril assembly, skeletal muscle contraction, ventricular cardiac muscle tissue morphogenesis, transition between fast and slow fiber, regulation of the force of heart contraction, sperm motility and chemical synaptic transmission.

Furthermore, in KEGG pathway analysis, DEGs were significantly enriched in olfactory transduction, cardiac muscle contraction, adrenergic signaling in cardiomyocytes, neuroactive ligand–receptor interaction, dilated

Figure 2. Mutation of *TP53* is associated with prostate cancer progress and prognosis. (A) Correlation between *TP53* mutation and mRNA expression. **(B)** Correlation between *TP53* mutation and Gleason score. **(C)** Kaplan–Meier survival curves for prostate cancer patients stratified by *TP53* mutation.

*** $p < 0.0001$

TCGA: The Cancer Genome Atlas.

cardiomyopathy, salivary secretion, arachidonic acid metabolism, amphetamine addiction, mineral absorption, hypertrophic cardiomyopathy, cholinergic synapse and glutathione metabolism (Figure 5B).

Module screening from the PPI network

We screened the information in the STRING database to investigate the interaction and hub genes of DEGs. Ranked by degree, we identified the top ten genes as hub genes. These genes were *ACTA1*, *ACTN2*, *ACTC1*, *TNNC1*, *MYH7*, *MYL1*, *MYH6*, *AVP*, *MYL3* and *TCAP*. *ACTA1* was identified as the top hub gene with the degree of 31. We then imported the analyzed network into Cytoscape software to identify modules of genes using the MCODE plate. MCODE helped us find out the top three modules. Then, we did enrichment analysis on the top three significant modules (Supplementary Table 1 & Figure 6, respectively). Results showed that functional annotation in module 1 genes was primarily enriched in muscle filament sliding, cardiac muscle contraction, skeletal muscle contraction, ventricular cardiac muscle tissue morphogenesis, transition between fast and slow fiber. While in module 2, the analysis indicated the enrichment were mainly associated with chemical synaptic transmission and GPCR receptor signaling pathway, coupled to cyclic nucleotide second messenger.

Figure 3. Gene set enrichment analysis results of TP53 mutation in prostate cancer patients.

Discussion

TP53, containing transcriptional activation, DNA binding and oligomerization domains, acts as a tumor-suppressor protein in many tumor types. Until now, *TP53* mutation has been found to be universal across cancer types [17]. *TP53* has been shown to play critical roles in dealing with various cellular stresses to regulate target gene expression, which induces transcriptional activation, DNA repair, apoptosis, senescence, cell cycle arrest or changes in metabolism [4]. Mutations in *TP53* have been found in multiple human cancers, including prostate cancer, making it a potential biomarker for individualized treatment.

Figure 4. Volcano plot for differentially expressed genes.

Here in this study, we analyzed an RNA-Seq dataset of prostate cancer downloaded from TCGA to identify the key pathways and genes associated with *TP53* mutation using bioinformatics analysis approaches. We found 18% patients with *TP53* mutation among 499 cases, including truncating, deep deletion and missense mutations spanning over entire gene. Our results showed relatively lower expression level of *TP53*, higher Gleason score and poorer prognosis in patients with *TP53* mutations, suggesting that *TP53* mutation may play an important role in prostate cancer disease program. The abnormal regulation of *TP53* at the transcriptional level, as well as the mechanism of epigenetics, is the main mechanism known to cause deregulation of *TP53*, and our study provides a new possible mechanism for the abnormal expression of *TP53*. As shown in previous studies, lower *TP53* expression level often correlates to disease progression and worse prognosis in various cancer types, which suggests that the decreased gene expression caused by *TP53* mutation may be one of the potential mechanisms involved in the disease process. Therefore, our findings provide a novel basis for further study of the mechanisms of the pathogenesis and progression of prostate cancer [18,19].

To find out some other potential mechanisms how *TP53* mutation influences prostate cancer development, we then did GSEA. Results showed that *TP53* mutation was significantly associated with metabolism, DNA repair, mitosis and several signaling targets or pathways, such as E2F targets, MYC targets, mTORC1 signaling pathway, PI3K-AKT-mTOR signaling pathway and TGF- β pathway by GSEA. Previous studies have shown that metastatic, castration-resistant prostate cancer has genomic aberrations that interfere with DNA repair [20,21]. Poly(ADP-ribose) polymerase is involved in multiple aspects of DNA repair, and poly(ADP-ribose) polymerase inhibition has been shown to exert antitumor activity in sporadic cases of metastatic, castration-resistant prostate cancer by dealing with DNA-repair defects in tumor cells [22]. Otherwise, antiandrogen treatment, the core treatment strategy in prostate cancer, could cause increased DNA damage and decreased clonogenic survival, providing new insight in prostate cancer development mechanism. Metabolic processes, including glycolysis, fat and protein metabolism, always play quite critical role in cancer progression. Metabolic target has been shown to be a potential treatment target in prostate cancer [23]. Furthermore, E2F targets, MYC targets, mTORC1 signaling pathway, PI3K-AKT-mTOR signaling pathway and TGF- β pathway are all critical signaling molecules and pathways in tumor disease progression [24–26]. Our results suggest that *TP53* mutation was significantly associated with the cellular programs above, which implies the potential mechanisms how *TP53* mutation influences prostate cancer progression. For the concept precision medicine, we can further investigate whether drugs targeting signaling targets above may be more effective in patients with *TP53* mutation.

To further dig out some potential mechanisms and novel drug targets, we then identified the DEGs in both groups and carried out the GO term and KEGG analysis. Results show about 950 genes as DEGs with different mutation status of *TP53*. Enrichment analysis indicated that DEGs in *TP53* mutation prostate cancer patients

Figure 5. Database for annotation, visualization and integrated discovery enrichment results of differentially expressed genes. (A) The GO enrichment terms of differentially expressed genes. (B) The KEGG pathway analysis of differentially expressed genes.

HCM: Hypertrophic cardiomyopathy.

were related to biological processes of cell growth and metabolism, which implies that patients with *TP53* mutation might have more vigorous cell energy metabolism and cell growth. Previous studies have confirmed that adaptations across multiple metabolic processes are necessary to satisfy the energy required for increased rate of proliferation in the malignant transformation progression. Deregulation of cell metabolism, such as increased lipid accumulation, has been a hallmark of the malignant phenotype. Changes in the levels of a variety of lipid metabolic enzymes have been documented in prostate cancer, which also provide various treatment targets [27]. Therefore, analysis above suggests the function of *TP53* mutation in cellular progression and provides insight into the promising therapeutic target in patients with *TP53* mutation for individualized treatment opinions.

Figure 6. Top two modules from the protein-protein interaction network. (A) Protein-protein interaction network of module 1. (B) Protein-protein interaction network of module 2. (C) GO analysis of the top two modules.

In PPI network analysis, we identified hub genes that consisted of the ten DEGs with the highest degree of interaction. The *ACTA1* gene, which encodes alpha actin belonging to the actin family of proteins, regulates the fetal form of actin. *ACTA1* upregulation is associated with improved muscle function in certain myopathies. Other research showed that *ACTA1* gene is upregulated in prostate cancer patients on androgen-deprivation therapy (ADT), which may represent a compensatory response to ADT-induced muscle loss [28]. As we all know, prostate cancer is a kind of androgen-sensitive disease. Therefore, ADT is still the primary treatment for localized prostate cancer, which induces a biochemical and clinical response in the majority of patients. Our results link *TP53* mutation to ADT, suggesting that *TP53* mutation may play a potential role in drug therapy, drug resistance and progression to castration-resistant prostate cancer in prostate cancer. These aspects still require further research to confirm.

Conclusion

This study determined the main pathways and genes associated with the *TP53* mutation in prostate cancer, which may promote the development of *TP53* mutation to improve prostate cancer risk prediction and develop treatment strategies for male prostate cancer.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/bmm-2019-0141

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Ethical conduct of research

The authors state that this study was approved by the Ethics Committee of Nanjing Medical University and they have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

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Summary points

- Prostate cancer is the most common malignancy in men in developed countries. The *TP53* mutant is one of the most common mutant genes in primary prostate cancer, making it a potential biomarker for individualized therapeutic perspectives.
- *TP53* mutation was found in 18% of prostate cancer patients. A total of 950 genes were identified as differentially expressed genes (DEGs).
- GO and KEGG analysis indicated that DEGs were significantly enriched in the classes associated with GPCR signaling pathways, negative regulation of growth, olfactory transduction and glutathione metabolism.
- Our results showed lower expression level of *TP53*, higher Gleason score and poorer prognosis in patients with *TP53* mutations.
- Our findings have indicated that multiple genes and pathways may play a crucial role in *TP53* mutation prostate cancer, offering candidate targets and strategies for *TP53* mutation prostate cancer-individualized treatment.

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