Trp53 regulates platelets in bone marrow via the PI3K pathway

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Abstract. The p53 gene is well known as a key tumor suppressor gene; it is vital for hematopoietic stem cell differentiation and growth. In the present study, the change of platelets (PLTs) in p53 knockout mice ($p53^{-/-}$ mice) was investigated. The peripheral blood cell subsets and PLT parameters in $p53^{-/-}$ mice were compared with those in age-matched $p53^{+/+}$ mice. Bleeding time as well as the alteration of PLT levels, were analyzed with the PLT marker CD41 antibody using flow cytometry. The results revealed that the number of PLTs in $p53^{-/-}$ mice was significantly lower than that in $p53^{+/+}$ mice. Bleeding time was prolonged in the peripheral blood of $p53^{-/-}$ mice compared with that of $p53^{+/+}$ mice. Furthermore, the related gene expression of the PI3K signaling pathway in the bone marrow of $p53^{-/-}$ mice was shown to be associated with plateletogenesis. PI3K inhibitor (LY294002) was also used to treat $p53^{-/-}$ mice, and the results demonstrated that LY294002 revert the change of PLTs in these mice. In summary, PLTs were altered in $p53^{-/-}$ mice, and the PI3K signaling pathway was involved in that process, suggesting that the p53-dependent PI3K signaling pathway is involved in thrombocytopenia or PLT diseases. PLT number is reduced in p53 deficiency; however, this reduction could be reverted by inhibiting the PI3K pathway.

Introduction

p53 was first discovered in 1979, in a study of Simian Virus 40 (SV40), a virus oncoprotein (1,2). And in the same year, another research team reported the identification of its coding genes (3). Until 1989, p53 had been considered an oncogene; however, in later years, the wild-type p53 gene was identified as a tumor suppressor gene that inhibits tumor formation (4), and the activation of p53 was shown to be able to lead to cell cycle arrest and apoptosis (5).

p53 is one of the most important tumor suppressor genes, and its encoded protein is vital for maintaining the stability of numerous genes, and regulating cell growth, differentiation and aging, as well as preventing diseases (6). Therefore, it is also called the 'Police Gene' (7,8). Deletion or mutation of the tumor protein p53 gene has a direct impact on the functions and activities of p53 protein, leading to cancer occurrence. More than 50% of cancers, including hematological malignancies and solid tumors, are caused by mutations (4).

Platelets (PLTs) are components of mammalian blood derived from the cytoplasmic cleavage of megakaryocytes in the bone marrow, and possess a biologically active, rather than strict small cytoplasm (9). Megakaryocytes originally come from directional differentiation of multifunctional hematopoietic stem cells in the bone marrow, and further become mature megakaryocytes (10-12). The major functions of PLTs include coagulation, hemostasis and repairing damaged blood vessels; they prevent atherosclerosis by protecting and repairing vascular endothelium (13).

The knowledge of the underlying mechanisms of p53 in the development of hematological disease and cancer could have considerable prognostic and therapeutic values in clinical applications (14). The p53 gene has been used as a strong indicator in determining the prognosis of tumors, since its mutation is associated with cancer development (15). Previous studies have reported that p53 plays an important role in megakaryocyte differentiation and apoptosis (16,17). Knockdown of p53 increases ploidy of megakaryocytes, as indicated from *in vivo* and ex vivo studies in 2012 (18). p53 deficiency promotes polyploidization during megakaryopoiesis, suggesting a direct association between p53 loss and the development of fully functional megakaryocytes. However, the signaling pathways involved in p53 regulation of megakaryopoiesis and blood cell differentiation still remain poorly understood.

In the present study, the changes of PLT parameters in $p53^{-/-}$ mice were analyzed to investigate the manner in which p53 regulates PLT differentiation. The results revealed that the number of PLTs in $p53^{-/-}$ mice is significantly lower compared with that in wild-type mice, and that p53 regulates PLT formation via the PI3K signaling pathway *in vivo*, providing useful insight for the investigation of the molecular mechanisms

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underlying the differentiation of hematopoietic stem cells into megakaryocytes.

Materials and methods

Animals. p53 knockout mice(total number, 60; age, 18-20 weeks; weight, 20-25 g; 32 males and 28 females), referred to as $p53^{-/-}$ mice, and wild-type mice with a BL/6J background, were donated as gifts by Professor Tiebang Kang from Sun Yat-Sen University Cancer Center (Guangzhou, China). Littermates identified as the $p53^{+/+}$ genotype were used as the wild-type control. Mice were bred and kept under SPF conditions at the Animal Center of Guangdong Pharmaceutical University (Guangzhou, China). Feed treated with ^{60}Co irradiation for sterilization was purchased from the Guangdong Medical Animals Center. Drinking water was autoclaved and mice received free access to food and water. The room temperature was kept at 24±2˚C and humidity was maintained at between 40 and 60%. Noise level was <60 dB. All 18 to 20 week-old mice were sacrificed via cervical dislocation under ether anesthesia. The experiments on mice were approved by the Animal Ethics Committee of the Guangdong Pharmaceutical University.

Genotype identification. The genotypes of p53 mice were identified by PCR. DNA was extracted from mice via the tail. DreamTaq Green PCR Master Mix for PCR was purchased from Thermo Fisher Scientific, Inc. (cat. no. K1081). The wild-type primers used were as follows: Wild-type primer 1, 5'-CAGCGTGGTGGTACCTTAT-3'; and wild-type primer 2, 5'-CTATCAGGACATAGCGTTGG-3', with a PCR product size of 450 bp. The mutant primers used were as follows: Mutation primer 3, 5'-TATACTCAGAGCCGGCCT-3'; and mutation primer 4, 5'-CTATCAGGACATAGCGTTGG-3', with a PCR product size of 615 bp. PCR conditions were as follows: Denaturation at 94˚C for 3 min, 94˚C for 1 min, 60˚C for 2 min and 72˚C for 2 min, for 30 cycles, followed by extension at 72˚C for 5 min. Gel electrophoresis was performed using 1.2% agarose gel. Images were captured with an automatic gel imaging system (Syngene).

Blood collection and routine blood tests. Blood was collected from the mice (18-20 weeks old). Blood cell subtypes were detected at the Guangdong Laboratory Animal Monitoring Institute using the blood cell counter XT-2000i (Sysmex Corp). Ether was used for anesthesia.

PLT preparation. PLTs were isolated from the whole blood of the mice (19). Briefly, 6:1 of citrate-dextrose solution (Sigma-Aldrich; Merck KGaA) and whole blood were gently mixed and centrifuged at 280 x g for 15 min at room temperature. The PLT-rich plasma (PRP) was obtained by another centrifugation at 400 x g for 5 min at room temperature. To obtain PLT pellets, PRP was further centrifuged at 1,100 x g for 10 min at room temperature and the PLT pellets were suspended in PBS for immediate use. In the indicated experiments, PLTs were stained with CD41 antibody and visualized under a fluorescence microscope or analyzed by flow cytometry.

Flow cytometry. Blood samples of 18-20 weeks old $p53^{-/-}$ and wild-type mice were subjected to flow cytometry analysis. Fresh blood was taken from ether anesthesia, and then added into anticoagulant tubes. A total of 10 μl blood was added into labeled flow tubes and mixed with 90 μl PBS. The mixture was incubated with antibody CD41-PE [BG-03512-60-100; mouse IgG1, κ-isotype; BioGems, Ltd. 1:100 with 1% BSA (Sangon Biotech Co, Ltd. cat. no. A600332)] at room temperature in the dark for 30 min. Finally, 1 ml 1% pre-cooled paraformaldehyde was added and the fixed mixture was preserved at 2-8˚C in the dark (20). The samples were gated to exclude irrelevant constituents, such as lymphocytes and cell debris before analysis. The positive rate of the specimens and the intensity of fluorescence were measured. BD FACSCanto™ II system (BD Biosciences) was used for analysis. EDTA-K2 anticoagulant was used in blood collection. After being treated with ACD anticoagulant (C3821; Sigma Aldrich; Merck KGaA), the blood samples were used to analyze the PLTs by flowJo 7.6.1 (FlowJo LLC).

Immunofluorescence. For immunofluorescence staining, bone marrow and blood smearing sections were fixed with 1% paraformaldehyde at 4˚C for 25 min and stained with the PLT marker CD41-PE for 30 min (BG-03512-60-100, mouse IgG1, κ-isotype; BioGems, Ltd. dilution:1:100 with 1% BSA) at room temperature, and then the sections were counterstained with DAPI (100 ng/ml) for 10 min at room temperature in the dark and all sections were visualized under a fluorescent microscope (BX53 Olympus microscope; Olympus, Corp).

Bleeding time test. According to the method utilized in previous studies (21,22), a 50 ml glass centrifuge tube filled with sterile isotonic saline was pre-warmed in water bath at 37˚C for 30 min. Next, 3 mm of the tail of each anesthetized mouse was cut with a scalpel and immediately placed in physiological saline. Bleeding time was recorded as the time from the moment bleeding started until bleeding had stopped, for a minimum of 10 sec. The assay was automatically stopped if the total bleeding time exceeded 20 min.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from PLTs in bone marrow using TRIzol®, and was quantified by the NanoDrop nucleic acid protein analyzer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). Reverse transcription was carried out according to the following protocol: 42˚C for 2 min, stored at 4˚C; 37˚C for 15 min and 84˚C for 5 sec, storage at 4˚C with the Reverse PrimeScript RT reagent kit (RR047A, Takara Bio Inc.). All gene primers purchased from Sangon Biotech Co. Ltd, which are listed in Table SI. The PCR kit was purchased from Thermo Fisher Scientific, Inc. The PCR and specificity of the primers were evaluated based on the standard curve of amplification. PCR volume was comprised of 10 μl SYBR Green Master Mix, 1 μl upstream primer and 1 μl downstream primer, 1 μl target cDNA and 7 μl ddH_2O. Reaction conditions were as follows: Pre-denaturation at 94˚C for 5 min, denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec, for 40 cycles, followed by extension at 72˚C for 10 min. The results were analyzed based on the dissolution curve. The Cq value of the threshold cycle was measured to calculate $2^{-\Delta\Delta Cq}$ and determine the expression levels of the target genes (23).

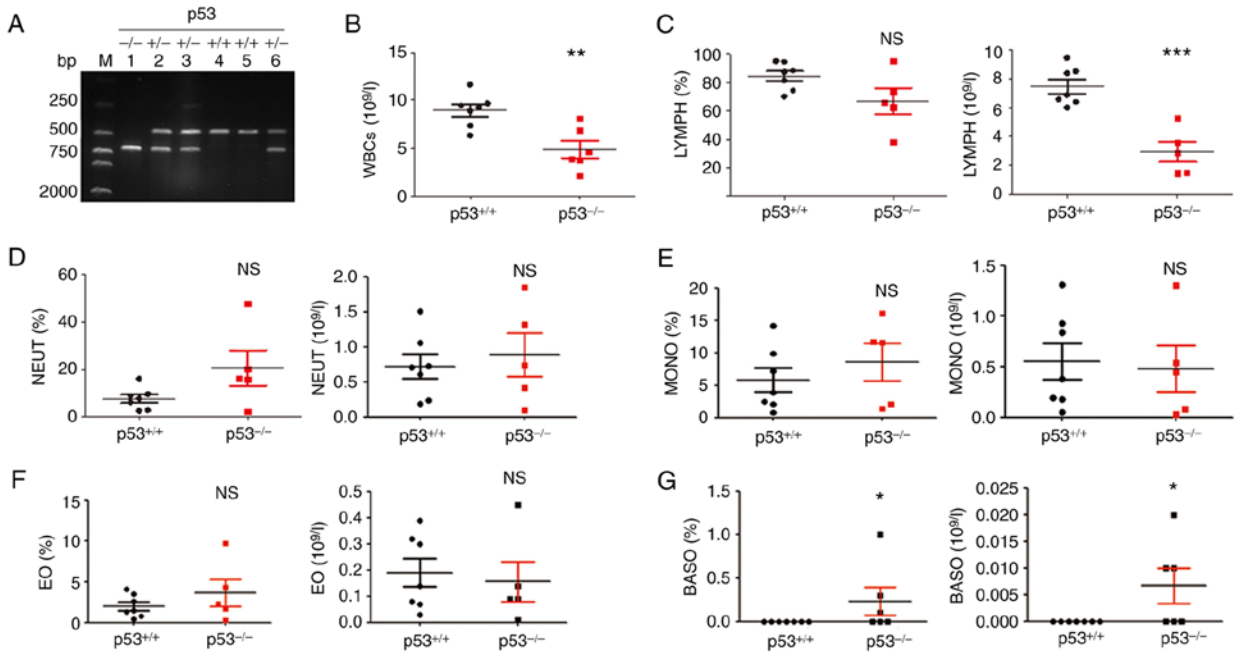


Figure 1. Genotype identification and routine blood test results. (A) p53 knockout mice was identified by PCR. Genotypes of $p53^{-/-}$, $p53^{+/-}$ and $p53^{+/+}$ mice are shown. The PCR product size of the wild-type mice was 450 bp, whereas that of the $p53^{-/-}$ mice was 615 bp. (B) Absolute counts of white blood cells. (C) Percentage of lymphocytes (left) and number of lymphocytes (right). (D) Percentage of neutrophils (left) and number of neutrophils (right). (E) Percentage of monocytes (left) and number of monocytes (right). (F) Percentage of eosinophils (left) and number of eosinophils (right). (G) Percentage of basophils (left) and number of basophils (right). $p53^{+/+}$ mice, n=6; $p53^{-/-}$ mice, n=6. *P<0.05, **P<0.01 and ***P<0.001. NS, no significance; EO, eosinophils; MONO, monocytes; BASO, basophils; NEUT, neutrophils; WBC, white blood cell; LYMPH, lymphocyte.

Administration of PI3K inhibitor. The 18-20 week mice were administered with a PI3 K inhibitor (LY294002, cat. no S1105; Selleck Chemicals). Starting from 20 weeks, a short-term assay was used with intraperitoneal administration of ≥200 μl dose of PI3 K inhibitor (15 μg/g body weight; stock concentration: 50 mg/ml; needle gauge, 25G) each per day, for 7 consecutive days.

Statistical analysis. All data were analyzed using GraphPad Prism 5.0 software (GraphPad Software, Inc.) and presented as the mean ± standard deviation. A two-tailed t-test or one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test, was used for the comparison of the results and the evaluation of the statistical differences between the groups. *P<0.05 was considered as statistically significant; **P<0.01 and ***P<0.001.

Results

Identification of genotype and routine blood tests. $p53^{+/+}$, $p53^{+/-}$ and $p53^{-/-}$ mice were obtained by crossbreeding $p53^{+/-}$ male mice with $p53^{+/-}$ female mice. The size of the PCR product in the $p53^{-/-}$ mice was 615 bp, whereas that in the wild-type ($p53^{+/+}$) mice was 450 bp. The presence of both bands indicated heterozygous $p53^{+/-}$ mice. The mouse designated as 1# was a homozygote $p53^{-/-}$ mouse (Fig. 1A), the mice designated as 2#, 3# and 6# were heterozygous $p53^{+/-}$ mice, and the mice designated as 4# and 5# were homozygote $p53^{+/+}$ mice. These results were representative.

To explore whether blood cell subsets were altered in $p53^{-/-}$ mice, routine blood tests were performed. Results showed that the absolute count of white blood cells was decreased in $p53^{-/-}$ mice compared with that in $p53^{+/+}$ mice (**P<0.01; Fig. 1B). Lymphocytes percentage showed no significant difference; however, lymphocyte absolute number was decreased compared with that in $p53^{+/+}$ mice (***P<0.001; Fig. 1C). In addition, there were no significant differences in the percentage and absolute number of neutrophils, monocytes and eosinophils (Fig. 1D-F). The percentage and absolute number of basophils were significantly higher in $p53^{-/-}$ mice than those in the control (*P<0.05; Fig. 1G). There was no difference in the number of red blood cells, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin concentration and other red blood cell parameters between the two groups (data not shown).

PLT number and PLT parameters are altered in $p53^{-/-}$ mice. PLT counts an important diagnostic index for the clinical diagnosis and treatment of thrombocytopenia of various causes (24). The analysis of the blood cell test results by a blood cell counter demonstrated a significant alteration in the number of PLTs in the peripheral blood of $p53^{-/-}$ mice, as shown in Fig. 2. The PLT count and plateletcrit (PCT) of $p53^{-/-}$ mice were significantly lower than those of wild-type mice (**P<0.01; Fig. 2A and B). The PLT-large cell ratio (P-LCR) was significantly increased (Fig. 2C; *P<0.05). Nevertheless, there was no significant change in PLT distribution width (Fig. 2D). Mean PLT volume showed an increased tendency in $p53^{-/-}$ mice; however, there was no significant difference compared with the control group (wild-type mice, n=6; $p53^{-/-}$ mice, n=6; Fig. 2E).

CD41-positive PLTs are decreased in the peripheral blood of $p53^{-/-}$ mice. As aforementioned, the number of PLTs in $p53^{-/-}$ mice was significantly lower compared with that in wild-type mice. To investigate the function of PLTs, the bleeding time test was performed. The results indicated that the bleeding

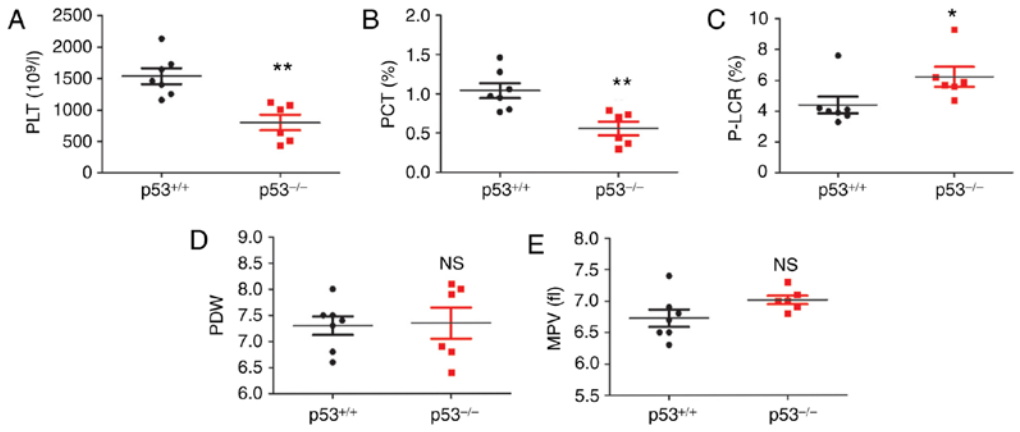


Figure 2. Changes of the PLT parameters in p53$^{-/-}$ mice. (A) Absolute PLT. (B) PCT. (C) P-LCR. (D) PDW. (E) MPV (n=6 per group). *P<0.05 and **P<0.01; NS, no significance; PLT, platelet; PCT, plateletcrit; P-LCR, PLT large cell ratio; PDW, PLT distribution width (%); MPV, mean PLT volume.

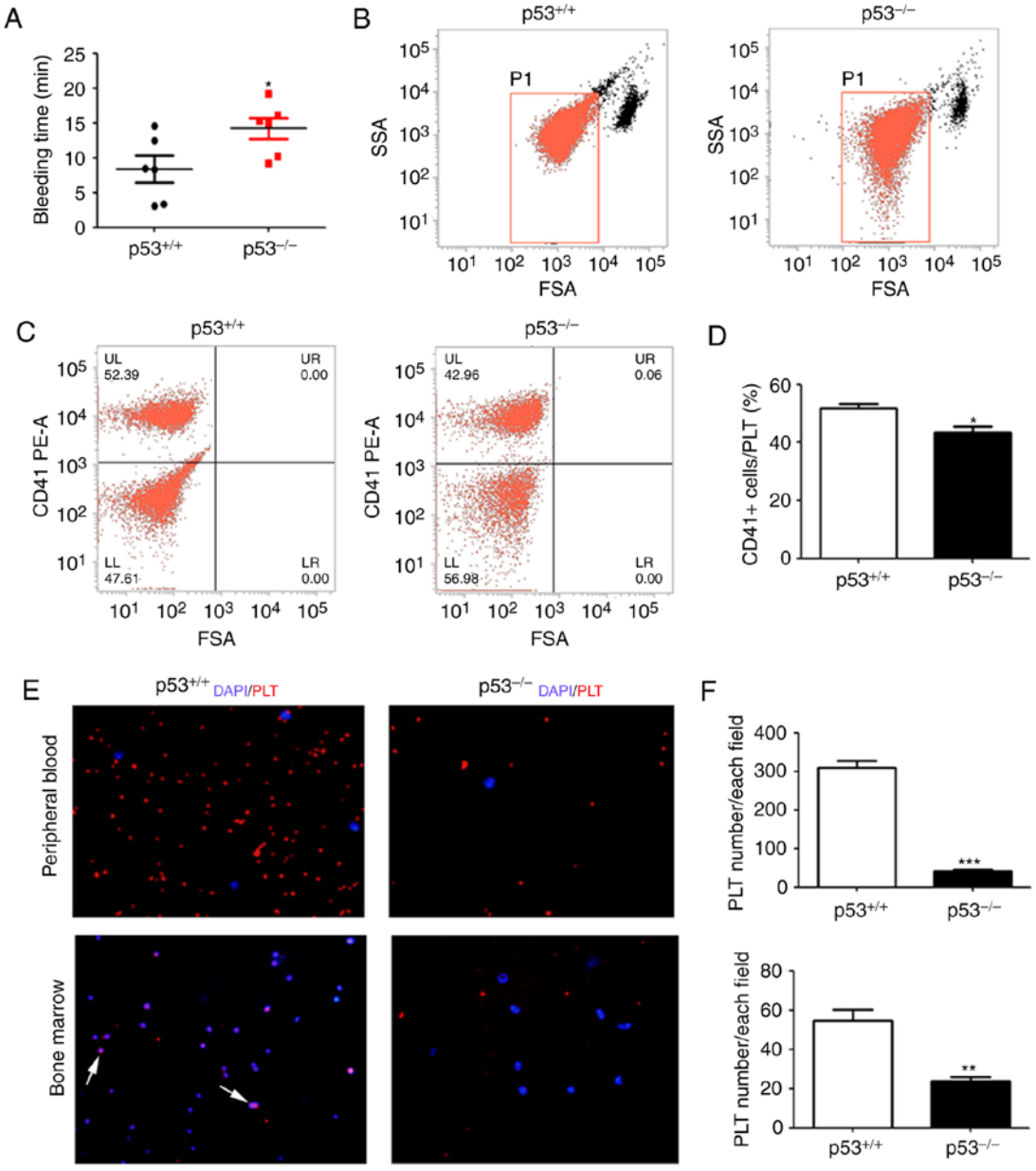
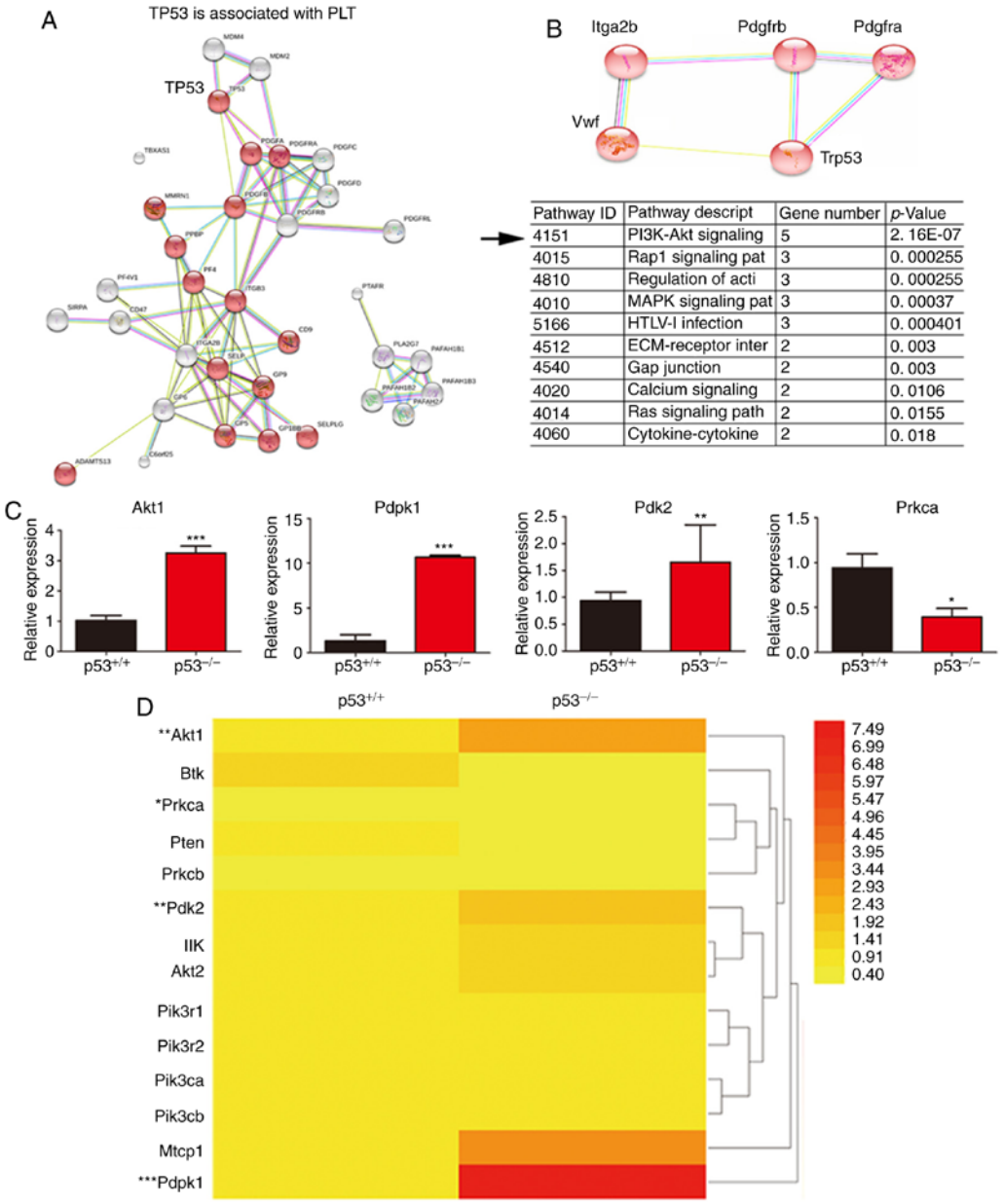


Figure 3. Bleeding time and CD41 positive PLTs in peripheral blood and bone marrow. (A) Bleeding time in p53$^{-/-}$ and p53$^{+/+}$ mice. (B) Gating of PLTs in peripheral blood of both groups of mice. (C) Percentage of CD41 positive cells in peripheral blood of both groups of mice. (D) Bar graph of the percentage of CD41 positive cells. (E and F) Immunofluorescence of the blood and bone marrow with CD41 antibody in p53$^{-/-}$ and p53$^{+/+}$ mice (magnification x400). (F) Quantitative assessment the data presented in E. n=6 per group. *P<0.05, **P<0.01 and ***P<0.001. PLT, platelet; FSE, forward scatter; PE, phycoerythrin.



Pathway ID	Pathway descript	Gene number	*p*-Value
4151	PI3K-Akt signaling	5	2. 16E-07
4015	Rap1 signaling pat	3	0. 000255
4810	Regulation of acti	3	0. 000255
4010	MAPK signaling pat	3	0. 00037
5166	HTLV-I infection	3	0. 000401
4512	ECM-receptor inter	2	0. 003
4540	Gap junction	2	0. 003
4020	Calcium signaling	2	0. 0106
4014	Ras signaling path	2	0. 0155
4060	Cytokine-cytokine	2	0. 018

Figure 4. Bioinformatics analysis and detection of PI3K pathway. (A) Detection of p53 and other genes related to PLTs through online bioinformatics analysis (https://string-db.org/cgi/input.pl). The p53 gene is shown to be closely related to PLT associated factors. (B) Data analysis showed that the PI3K signaling pathways involved in the regulation of p53 and PLTs. (C) Expression of genes related to the PI3K signaling pathwayin the bone marrow of $p53^{-/-}$ and $p53^{+/+}$ mice as assessed by RT-qPCR. The PI3K pathway is associated with p53 deficiency, which is consistent with the results of the bioinformatics analysis. (D) Heat map showing that the expression levels of few genes were altered in the PI3K signaling pathway in p53-deficient mice. Including Akt1, Prkca, Pdk2, and Pdpk1. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$. RT-qPCR, reverse transcription-quantitative PCR; PLT, platelet; Akt1, AKT serine/threonine kinase 1; Prkca, protein kinase C alpha; Pdk2, pyruvate dehydrogenase kinase 2; Pdpk1, 3-phosphoinositide dependent protein kinase 1.

time of $p53^{-/-}$ mice was prolonged compared with that of the control ($^{*}P<0.05$; Fig. 3A). To further validate the alteration in PLTs, flow cytometry was conducted for the detection of PLTs, using the CD41 antibody (as a PLT-specific marker) which is used to specifically identify PLTs and detect changes in their quantity (25). The peripheral blood of $p53^{-/-}$ and wild-type mice were labeled and analyzed by flow cytometry. Next, a group of cells was gated between lymphocytes and cell debris (26), as shown in Fig. 3B. The results showed that the percentage of CD41-positive cells in the $p53^{-/-}$ group was significantly lower than that in the wild-type mice ($^{*}P<0.05$; Fig. 3C and D). The results of flow cytometry were consistent with the routine blood test results obtained by an automatic blood cell counter. The results demonstrated that the number of PLTs in $p53^{-/-}$ mice was significantly lower than that in the control group. Furthermore, CD41 was detected in the blood and bone marrow by immunofluorescence, and the results also showed that the PLT number in $p53^{-/-}$ mice was significantly decreased in the bone marrow and blood ($^{***}P<0.001$ and $^{**}P<0.01$; Fig. 3E and F).

PI3K signaling pathway is changed in bone marrow of $p53^{-/-}$ mice. p53 knockout in mice with changes in PLTs indicated that certain proteins involved in PLT production were altered. Therefore, 'p53' and 'platelet' were chosen as keywords for the protein and protein action analysis via the biology information website (http://version10.string-db.org/). The association of

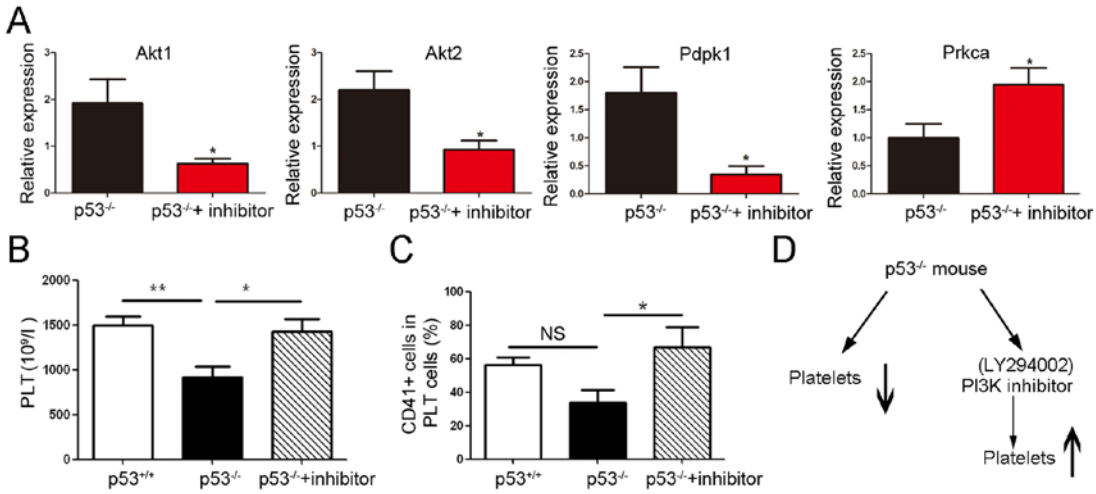


Figure 5. PLT number was increased in p53$^{-/-}$ mice treated with PI3K inhibitor. (A) Administration of PI3K inhibitor revert the alterations of Akt1, Akt2, Pdpk1 and Prkca mRNA expression levels. (B) After treatment with PI3K inhibitor (LY294002), the PLT count was found to be increased in the blood of p53$^{-/-}$ mice using a blood cell counter (here, n=5 per group; 18-20 weeks old). (C) CD41 was used as a PLT biomarker to detect the PLTs after treatment with PI3K inhibitor by flow cytometry. PLT count of p53$^{-/-}$ mice administrated with the inhibitor was significantly increased compared with that of the control group. (D) Schematic diagram showing that the administration of PI3K inhibitor could revert the alterations in the PLTs derived from p53$^{-/-}$ mice. *P<0.05, **P<0.01. NS, no significance; PLT, platelet.

p53 and molecules related to PLTs, including integrin subunit alpha 2b, von willebrand factor, platelet derived growth factor receptor alpha and platelet derived growth factor receptor beta, were analyzed and confirmed (Fig. 4A). Next, the key genes involved in PLT production and p53-related signaling pathways were further analyzed. The results suggested that the PI3K signaling pathway may be a bridge linking p53 and PLT-related factors (***P<0.001; Fig. 4B). This conclusion is consistent with findings from a previous study (18). To further confirm whether p53 regulates PLT production through the PI3K signaling pathway, total RNA was extracted from the bone marrow of p53$^{-/-}$ and wild-type mice. In this pathway, Akt1 (AKT Serine/Threonine Kinase 1), Pdk2 (pyruvate dehydrogenase kinase 2) and Pdpk1 (3-phosphoinositide dependent protein kinase 1) mRNA levels were increased in the bone marrow of p53$^{-/-}$ mice, where Prkca (protein kinase C-α) mRNA expression level was decreased, suggesting that the PI3K signaling pathway is involved in p53 knockout (*P<0.05, **P<0.01 and ***P<0.001; Fig. 4C). In conclusion, the expression levels of the PI3K pathway-related genes and the heat map of these genes suggest that the PI3K pathway may change in the bone marrow of p53$^{-/-}$ mice (Fig. 4D).

PI3K inhibitor reverts the PLT downregulation in p53$^{-/-}$ mice. To investigate whether the inhibitor of PI3K (LY294002, cat. no. S1105; Selleck Chemicals) could revert the changes in PLTs in p53 knockout mice, a short-term assay was conducted with intraperitoneal administration of a dose of PI3K inhibitor once per day for 7 consecutive days. Akt1, Akt2 (AKT Serine/Threonine Kinase 2), Pdpk1 and Prkca mRNA expression levels were determined by RT-qPCR, and the results revealed that the Akt1, Akt2 and Pdpk1 in PI3K signaling pathway were suppressed; however, the Prkca mRNA level was increased (*P<0.05; Fig. 5A).

Next, the changes in PLT numbers were assessed using the blood cell counter XT-2000i, and the CD41-positive PLTs were further detected by flow cytometry. The results revealed that PLT count was increased in p53$^{-/-}$ mice treated with PI3K inhibitor (ANOVA followed by Dunnett's test, *P<0.05 and **P<0.01; Fig. 5B and C), suggesting that the reduction in PLTs was reverted by the PI3K inhibitor (Fig. 5D).

Discussion

p53 was originally regarded as an oncogene; however, later on, it was designated as a tumor suppressor gene maintaining genome stability. A previous study has shown that p53 expression is high in megakaryocytes (27). Further attention has been paid to the role of p53 in hematopoietic stem cell differentiation (28). p53 has been found to regulate the differentiation of megakaryocytes and macrophages (29-31), specifically mediated megakaryocytic polyploidization and apoptosis (16). A study published in 2012 has shown that PLT count in p53$^{-/-}$ mice is slightly lower than that in wild-type mice, indicating that p53 could alter PLT function in p53-deficient mice (18). In the present study, a significant reduction in PLTs and PCT was observed in the blood of p53$^{-/-}$ mice, whereas an increase in P-LCR was presented. Blood cell counter XT-2000i was used for routine blood tests. The current veterinary software consists of settings and algorithms for the analysis of specimens from rats, mice and rabbits, which may explain the fact that PLT was slightly lower in p53$^{-/-}$ mice (18), whereas in our study it was observed to be significantly decreased. The results of the bleeding time test also supported the alteration of the PLT functions, in accordance with what has been previously reported (18).

The mRNA expression of genes is involved in the PI3K pathway in the bone marrow of p53$^{-/-}$ mice, as shown by bioinformatics analysis, and further confirmed with RT-qPCR. Of course, the protein of p-Akt should be detected by western blotting analysis; however, western blotting analysis was not performed in the present study, as there are only few megakaryocyte in bone marrow, and this experiment would require the use of additional mice. The expression of the related genes

PI3K, Akt1, Pdpk1 and Pdk2 was increased in the bone marrow of the $p53^{-/-}$ mice. The aim of the present study was to identify the association between these factors and PLT formation, and overexpression or silencing experiments were required for this purpose. However, the generation of PLTs is very complex in the bone marrow of mice, therefore, these experiments were not performed in the present study.

PI3K inhibitor was administered to $p53^{-/-}$ mice. Akt1, Akt2 and Pdpk1 mRNA expression levels were decreased, whereas the number of PLTs was shown to be increased. PLT-derived growth factor receptor and signaling pathways of PLT have been shown to be regulated by the PI3K pathway (32,33), which supports the present findings that p53 deficiency may down-regulate PLTs via the PI3K signaling pathway. In the present study, it was confirmed that these genes down-regulate PLTs and that PLT count could be reverted after the administration of PI3K inhibitor in p53 knockout mice. There are probably two reasons for the increase in PLTs by PI3K inhibitor; one depends on p53, and the other is that PI3K directly controls plateletgenesis. A previous study has shown that the PI3K pathway is associated with PLTs (34).

In general, PLTs are derived from megakaryocytes in the bone marrow. However, a recent study has demonstrated that megakaryocytes in lung tissue could also produce PLTs, arousing more research interest in PLTs from the lung (35). It would be interest to compare the changes in PLTs from the lung between p53 knockout mice and wildtype mice.

p53 has been shown to regulate macrophage differentiation (30), indicating that p53 could affect myeloid cell differentiation. Herein, we speculated that p53 deficiency could induce megakaryocyte abnormalities, leading to changes in PLT count and other PLT parameters. A significant decrease in PLT count and abnormalities in other PLT indices were observed as a result of p53 mutation. Although activation and aggregation of PLTs were not detected, the change of PLT parameters and bleeding time was useful to gain insight into p53 function in PLTs. The molecular mechanisms by which p53 induces alterations in PLTs require further investigation. It would be great significance to investigate the mechanism by which p53 regulates PLTs via the PI3K pathway in order to prevent PLTs from promoting tumor metastasis (36).

In conclusion, the present study results suggest that p53 could alter PLT number, bleeding time and that the PI3K signaling pathway is involved in this process, thus providing useful insights into the study of thrombocytopoiesis and controlling the amount of PLTs in the development of cancer. p53 is also useful for investigating the manner in which PLTs affect tumor metastasis or PLT diseases.

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Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Authors' contributions

JL and LW conceived and designed the study. MY, QL and TN acquired, analyzed and interpreted the data. JK, XZ, SL and XH were responsible for the sample collection and treatment. MY and LJ performed the statistical analysis. MY wrote the manuscript and revised it critically. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments on mice were approved by the Animal Ethics Committee of the Guangdong Pharmaceutical University (Guangzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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