THE REGULATORY EFFECTS OF Purα ON PARP1 GENE EXPRESSION AND ITS FUNCTIONS ON DNA REPAIR

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Abstract: A The luciferase reporter construct, which contains a polyADP-ribose polymerase 1 gene promoter, was transfected into U87MG cells with the Purα eukaryotic expression vector, and the activity of the PARP1 promoter was assessed by luciferase assay to evaluate the regulatory effects of Purα on PARP1 gene expression. The Purα eukaryotic expression vector was transfected into U87MG cells, and the cell total RNA and protein were extracted to determine the effects of Purα on PARP1 gene expression at transcriptional and translational levels by real time PCR and Western blot assay. The results demonstrated that Purα can positively regulate PARP1 promoter activity and promote PARP1 gene expression both at transcriptional and translational levels. Further study illustrated that Purα can collaborate with PARP1 in the repair of damaged DNA; the results of a pull-down assay suggested that there is a physical interaction between Purα and PARP1. The overexpression of Purα can increase endogenous PARP1 expression and alleviate the expression of the DNA damage signal protein, γH2AX. Above all, we believe that Purα possesses a positive regulatory effect on PARP1 gene expression and collaborates with PARP1 to repair damaged DNA.

Keywords: Purα eukaryotic expression vector, PARP1 promoter, PARP1 gene expression, damaged DNA

INTRODUCTION The maintenance of genomic DNA integrity is essential for cells to exert their normal functions. Exogenous factors such as ionizing radiation, UV light, and chemotherapeutic agents, can affect the integrity of the genetic materials. Endogenous processing, including oxidative metabolism, stalled DNA replications, and V(D)J recombination also produce DNA damage that disrupts the integrity of the genetic materials. These genotoxic agents/events can create single-stranded DNA breaks (SSBs) or double-stranded DNA breaks (DSBs). Without timely and accurate repair, DSBs represent a critical injury for cells that will result in gene mutations and genomic instability, as well as the loss or recombination of cellular chromatin DNA. The changes caused by the DSBs lead to cell apoptosis or oncogenesis [1]. Much attention is currently being paid to the reaction of cells to the DNA damage in proliferating cells and to tumor development; [1] however, comparatively little is known about the detailed effects of these processes on the nervous system. A proper cellular signaling reaction in response to DNA damage and the capacity to repair the damage is essential for the life of all organisms. Cells have a variety of DNA repair pathways to protect against damage [2].

The genes associated with DNA repair belong to the category of susceptibility genes, and polymorphism of these genes is common in organisms. This polymorphism can change the ability of individuals to repair DNA damage, and a decline or deficiency of repair ability may lead to instability of genomic DNA or oncogenesis. DNA damage-induced neurotoxicities have been associated

with the pathogenesis of many degenerative diseases of the nervous system, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. The key point at which the DNA damage participated in the genesis and development of the disorders and the mechanisms involved remain unclear [3]. In addition, recent studies have demonstrated that variants and polymorphisms of repair genes associated with DSBs, such as Ku80, RAD51, XRCC5, and XRCC1, are closely associated with high risks for oral cancer, glioma, hepatocellular carcinoma, and colorectal cancer [4-7]. A deficiency in the expression or the inactivation of an expressed product can also induce the instability of genomic DNA and is considered as the main reason for the genesis of breast cancer [8]. Poly(ADP-ribose) polymerase-1 (PARP1) is a nucleic enzyme that is located in the nucleus of eukaryotic cells and catalyzes polyADP-ribose polymerization. PARP1 is the main isoform of the 18 PARP family members. It consists of 1014 amino acids and has a molecular weight of 116 kDa. The encoding gene is located on chromosome 1q41～q42 and is composed of 23 exons, with an isoelectric point of 8.0～9.8 [9]. PARP1 is a highly conserved intra-nuclear DNA binding protein and can recognize a low level of DNA damage, activate the DNA repair system, and repair damaged DNA. When DNA damage is very serious, PARP1 can initiate cellular apoptosis to maintain the integrity of the genomic materials of the cell [10].

Since ADP-ribose polymerization can regulate the reaction to DNA damage by inhibiting the PAR induced by DNA damage, it is more sensitive to genetic toxicity for tumor cells, especially tumor cells with a deficiency in DNA repair. Therefore, PARP inhibitors have been designed and tested in tumor treatment [11-13]. Thus far, the treatment effects have been less than satisfactory. For example, the efficiency in a phase II clinical experiment of BRCA-defective tumors was only 40% [12].

Purα is an omnipresent nucleic acid-binding protein with the ability to bind to specific DNA sequences, which were originally located on the promoter of the mouse myelin basic protein gene. Based on these characteristics, it was purified from mouse brain tissue [14-15]. The Purα sequence is highly conserved among the different species and there are only 2 out of 322 amino acid residues that differ between the mouse and human beings [16-18]. As a multifunctional protein, Purα plays important roles in many body functions, especially in neurogenesis and development. Our previous work demonstrated that Purα could negatively regulate amyloid precursor protein gene expression, which could indirectly decrease amyloid β

formation and might be helpful in the prevention of Alzheimer's disease. The function of Purα in the survival and differentiation of neuronal cells has been proven in transgenic mice [19] and a model of Purα gene knockout mice has also been successfully established. Purα knockout (Purα$^{-/-}$) mice behaved normally at birth, but gradually developed neurological problems and displayed these abnormalities at the 15th postnatal day, which presented as serious tremors and unprompted seizures and finally death, inevitably by the 4th week after birth. Compared with the wild type age-matched littermates (Purα$^{+/+}$), the Purα$^{-/-}$ mice demonstrated abnormally small numbers of neurons in regions of the hippocampus and cerebellum, so it is not hard to speculate that Purα plays a critical role in neurogenesis. Abnormal chromosomes appeared in the Purα$^{-/-}$ cells when the cells were treated with genotoxic reagents. This suggested that Purα played an important role in DNA repair during neurodegeneration [20]. In addition, Purα played a critical role in monitoring the repair of DSBs induced by stalled replication forks [21].

Based on the above observations, we designed the current experiment. DNA damage is a detriment to each type of cell. It has been reported that Purα and PARP1 contribute to DNA damage and repair, respectively, implying that an interaction between Purα and PARP1 exists in DNA damage and coordinates the process of repair. However, the relationship between Purα and PARP1 still remains unclear with respect to how this relationship is involved in DNA damage and repair. What intrigued us was that as a multifunctional protein, Purα could upregulate the gene expression of PARP1. These results have not been previously reported, so far as we know. We performed a series of experiments to investigate the mechanism of this relationship to produce theoretical support for further study.

MATERIAL AND METHODS

Prokaryotic expression vectors pGEX-4T-1 (GE Healthcare Lifesciences, Marlborough, USA) and pGEX-4T-1-Purα, eukaryotic expression vectors pCDNA3.0 (Invitrogen, USA) and pCDNA3.0-Purα, reporter vector pGL3-basic (Promega, USA), Hela cell line, and human glioma cell line U87MG were all grown in our laboratory. Pierce® Glutathione Agarose and RevertAid First Strand cDNA Synthesis Kits were purchased from Thermo (USA). BugBuster® Maser Mix was purchased from Novagen (Germany). Commassie Blue Fast Staining Solution was purchased from Solarbio® (China). IPTG was obtained from Merck (Germany). RT-PCR Mix was purchased from CWBIO (China). Topo pCR2.1 cloning kits, Opt-MEM

medium, and lipofectamine2000 reagent were procured from Invitroge (USA). DMEM was obtained from HyClone (USA). Dual-Glo® Luciferase Assay System was purchased from Promega (USA). A BCA kit was from Keygene Biotech (China) and Rabbit anti-Purα Polyclonal Antibody (ab79936) and Rabbit Anti-γH2AX (phospho S139) Polyclonal antibody (ab2893) were purchased from Abcam (UK). Rabbit anti-PARP1 Polyclonal Antibody (TA332370S) was obtained from ORIGENE (USA). The Mouse Anti-β-actin monoclonal antibody (TA-09) was purchased from ZSBG-Bio Company (China).

Luciferase reporter assay

The control luciferase reporter plasmid, the constructed luciferase reporter plasmid for PARP1 pGL3-PARP1-500/+100, and the eukaryotic expression plasmid pCDNA3.0-Purα were transfected into U87MG cells together or separately. The U87MG cells were transfected with lipofectamine 2000 according to the manufacturer's instructions. At 48 hours after the transfection, cells were harvested for the experiment. In short, the culture medium was discarded, and the cells were washed with PBS, and then trypsinized. The trypsinized cell mix was

Primer	Sequence	Usage
Primer1-F	5'-CGGGG TACCG GAGGC	P PCR for
Primer1-R	TGAGG-3'	PARP1promoter
Primer4-F	5'- CCGGA GCTCG AGCGG	PCR for
	CGGAG-3'	PARP1promoter
	5'- AAGAC TCTGG GTGAC TTT-3'	RT-PCR for PARP1
Primer4-R	5'- CACCA TCTTG GACAG-3'	RT-PCR for PARP1
Primer4-F	5'- CGTGT TTATG CGAGT GAG-3'	RT-PCR for Purα
Primer4-R	5'- CCTCT GCTTC TCTTG AATC-3'	RT-PCR for Purα
Primer-F	5'- GAGTC AACGG ATTTG GTCGT-3'	RT-PCR for GAPDH
Primer-R	5'-GACAA GCTTC CCGTT CTCAG-3'	RT-PCR for GAPDH

Table 1. Primers for PCR and RT-PCR

Construction of a luciferase reporter plasmid containing the PARP1 gene promoter

Using the PARP1 gene sequence, we designed primers for the amplification of the PARP1 gene promoter. The promoter spans from -500 to +100, approximately 600 bps of the DNA sequence, on the proximal end of the PARP1 gene (Genbank, AL359704.9, 146087-146686). The primer sequences are listed in Table 1. To extract genomic DNA from U87MG cells, the synthesized primers were used to amplify the PARP1 promoter with high fidelity DNA polymerase *pfu*. We added deoxyadenosine to both ends of the amplified PCR fragment with *taq* DNA polymerase at 72° C for 30 minutes and then purified the fragment and ligated it with Topo pCR2.1 cloning vector. The positive colonies were selected using blue white screening and verified by sequencing. The appropriate fragment was cut with Kpn I and Xho I endonuclease and then purified and subcloned into the responsive MCS upstream of the luciferase gene in the reporter plasmid pGL3-Basic to construct the PARP1 promoter reporter plasmid pGL3-Basic-PARP1-500/+100.

spun at 1000 rpm for 5 minutes to collect the cell pellets and then 100 μl 1X lysis buffer was added to lyse the cells. The cell lysate was centrifuged at 15000 rpm for 10 minutes and the supernatant was separated for the luciferase activity analysis. To measure the luciferase activities, 50 μl of supernatant was added to 96-well plates. In addition, 50 μl Dual-Glo® Luciferase Reagent and Dual-Glo®Stop & Glo Reagent were added to each well and measured with a Centro LB 960. The luciferase assay was performed according to the instructions from Promega for the Dual-Glo® Luciferase Assay System. The activities of Renilla were used as an internal reference to adjust for transfection efficiency.

Real Time PCR (RT-PCR)

The Purα eukaryotic expression vector, pCDNA3.0-Purα, and the responsive control, pCDNA3.0, were transfected into U87MG cells with lipofectamine 2000. After the transfection (48 hours), the total cellular RNA was extracted with TRIzol. The extracted RNA was measured by a Nanodrop spectrophotometer to check the purity and quality. Reverse transcription was performed with a

RevertAid First Strand cDNA Synthesis Kit. The synthesized cDNA solution was diluted 10-fold, and 2 μl were used for Real Time-PCR in a Bio-Rad CFX96™ Real-Time System. The primers used for PCR were listed in Table 1, and GAPDH was used as an internal control.

Western blot assays
Purα eukaryotic expression vector, pCDNA3.0-Purα, SDS and the corresponding control, pCDNA3.0, were transfected into U87MG cells. After 48 hours, the cells were harvested for protein extract. The transfected cells were washed with ice-cold PBS and then lysed with RIPA buffer with protease inhibitor and phosphorylase inhibitor. The cell debris was removed by centrifugation and protein concentration was quantitated with BCA reagent. Furthermore, 40 μg of total protein was taken for SDS-polyacrylamide gel electrophoresis (PAGE). The protein was transferred to a PVDF membrane and blocked with 5% dry milk blocking buffer. The primary antibody was added, and the membrane was incubated at 4° C overnight. The next day, the membrane was washed with TBST buffer 5 times (5 minutes each time); the second antibody was added and incubated at room temperature for 60 minutes, then washed again as described above. The membrane was detected with an ODYSSEY CLx imaging system. The dilution of the primary antibody was 1:1000, it was 1:3000 for the internal control antibody β-actin, and it was 1:5000 for the fluorescent-tagged second antibody.

Pull down assay
To transform the recombinant plasmid of GST-Purα and the GST vector into competent BL21 cells, they were plated onto agar plates that contained ampicillin, and incubated at 37° C overnight. On the second day, a single clone was collected and put into 5 mL LB medium and allowed to grow overnight. The next day the culture was expanded to 100 mL LB medium for large-scale expression and purification of the target protein. Bacteria were incubated at 37° C in a shaking incubator until the culture had reached the mid-log phase of growth (OD_{590}=0.6). The target protein's expression was induced with IPTG (2 mM) at 29° C and 220 rpm. After the induction, the cells were grown for 4 hours and harvested by centrifugation at 4000 g for 5 minutes. Lysis buffer was added to resuspend the cell pellet (5 ml of lysis buffer per 100 ml of cell culture). The target protein was extracted based on the protocol from Novagen. Subsequently, 500 μl of Pierce® Glutathione Agarose was added to the protein extract. The mixture was shaken at 4° C overnight to adequately combine the target protein with Glutathione Agarose Beads. The mixture was centrifuged at 1000 rpm for 3

minutes to precipitate the Glutathione Agarose Beads. The beads were washed 20 times with cold PBS and a small amount of the supernatant was saved to determine the protein concentration and to analyze by SDS-polyacrylamide gel electrophoresis. Coomassie Brilliant Blue Staining was performed to evaluate the purity of the protein. After purification, the beads binding the protein were used to pull down its interacting protein from HeLa whole cell extracts (WCE). HeLa WCE (1 mg) was pre-cleared by GST beads and then bound with GST or GST-Purα beads. Binding proteins were eluted with NTN buffer containing 500 mM NaCl. The eluted proteins from GST beads and GST-Purα beads were separated on 4-20% SDS-PAGE, and GST-Purα was loaded as a control. The gels were stained with GelCode blue stain reagent. Many protein bands were presented on the GST-Purα lane. The strongest stained band was analyzed by mass spectrometry. The protein band was digested by trypsin and the digested peptide was analyzed using a MALDI-ToF/Pro (Amersham Biosciences) mass spectrometer and matched to peptide sequence information.

Statistical analysis
All data reported are the mean ± s.e.m ($\bar{x}$ ±S) and were analyzed using IBM software SPSS (version 20). One-way ANOVA was used for analyses. LSD method was performed to compare the means between multiple groups. For all analyses, α=0.05. Experiments were repeated at least 3 times.

RESULTS
Construction of a Luciferase reporter plasmid containing the PARP1 gene promoter
To identify the reporter plasmid, pGL3-PARP1, we used endonuclease cutting (Fig 1) and sequence analysis (Fig 2).
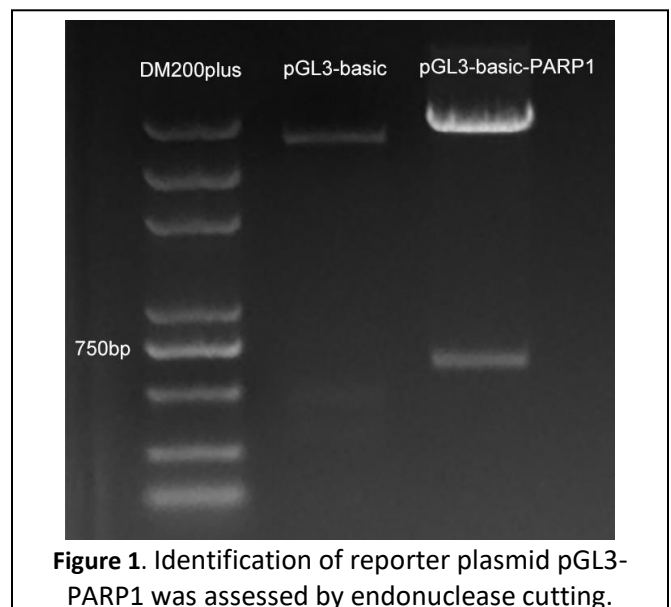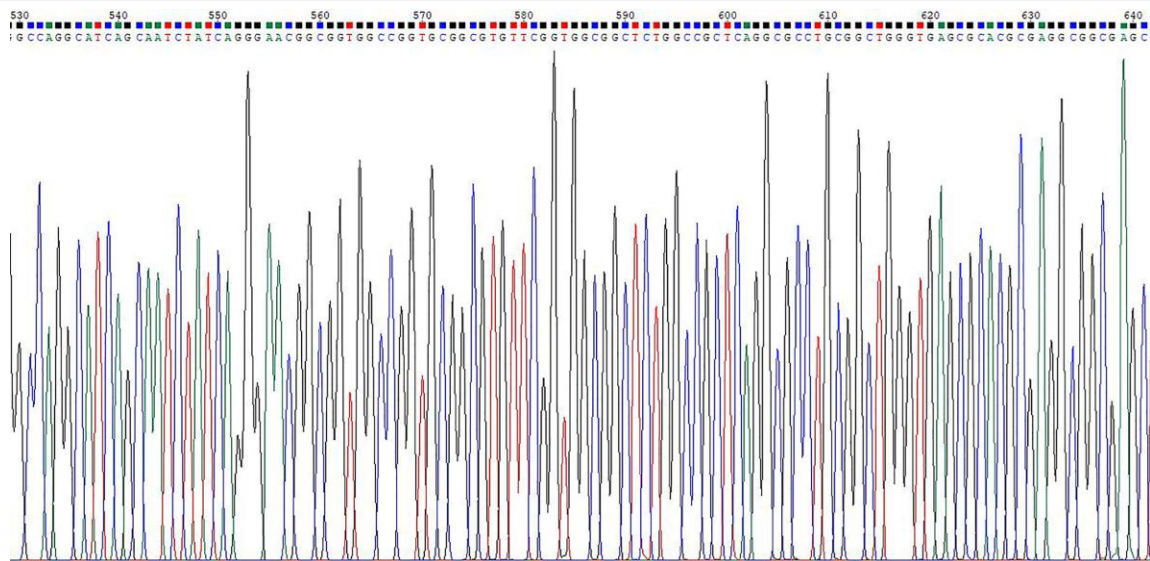

Figure 1. Identification of reporter plasmid pGL3-PARP1 was assessed by endonuclease cutting.

The band between 750 bp and 500 bp illustrated the band of 600 bp that we successfully inserted into the reporter plasmid, pGL3-PARP1. Meanwhile, the sequencing result demonstrated that the inserted fragment was a PARP1 promoter. The results are consistent with our experimental design. According to the luciferase assay, the activity of the constructed plasmid was sufficient for the experimental requirement.

much higher activity of the PARP1 gene promoter, which indicated that Purα significantly upregulated the activity of the PARP1 promoter (Fig 3).

To check the effects of Purα on PARP1 gene expression at the transcriptional level with qPCR
To further investigate the effects of Purα to regulate

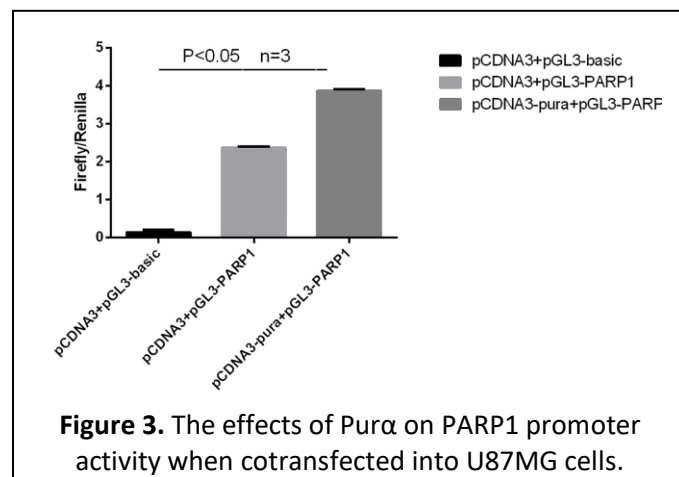

Figure 2. Identification of reporter plasmid pGL3-PARP1 was assessed by sequence analysis.

To evaluate the effects of Purα on PARP1 promoter activities with a Luciferase reporter assay
The constructed PARP1 reporter plasmid expressed great activity and was suitable for investigations of PARP1 gene expression. When transfected into U87MG cells, it had remarkable activity, while the empty reporter plasmid showed almost no activity with the luciferase assay. Purα illustrated up-regulatory effects on PARP1 gene expression when co-transfected PARP1 reporter plasmid in U87MG cells was compared with those co-transfected with pCDNA3 (control group). The Purα group expressed
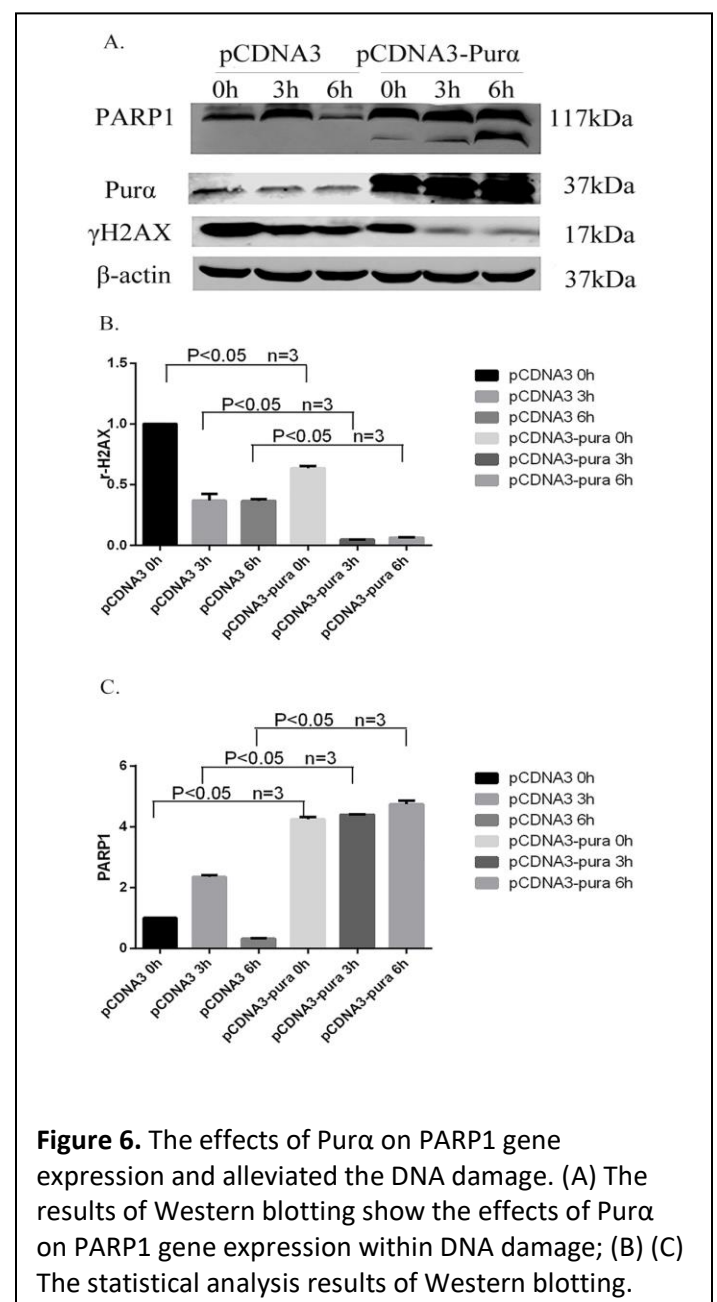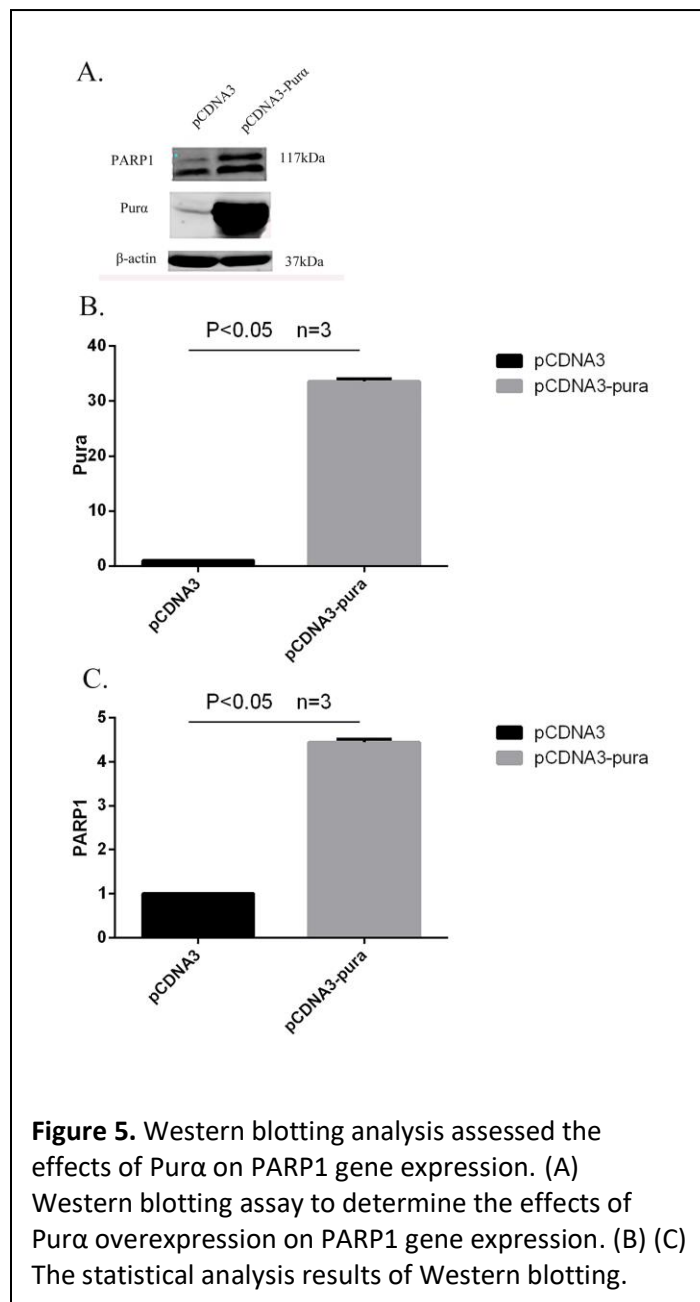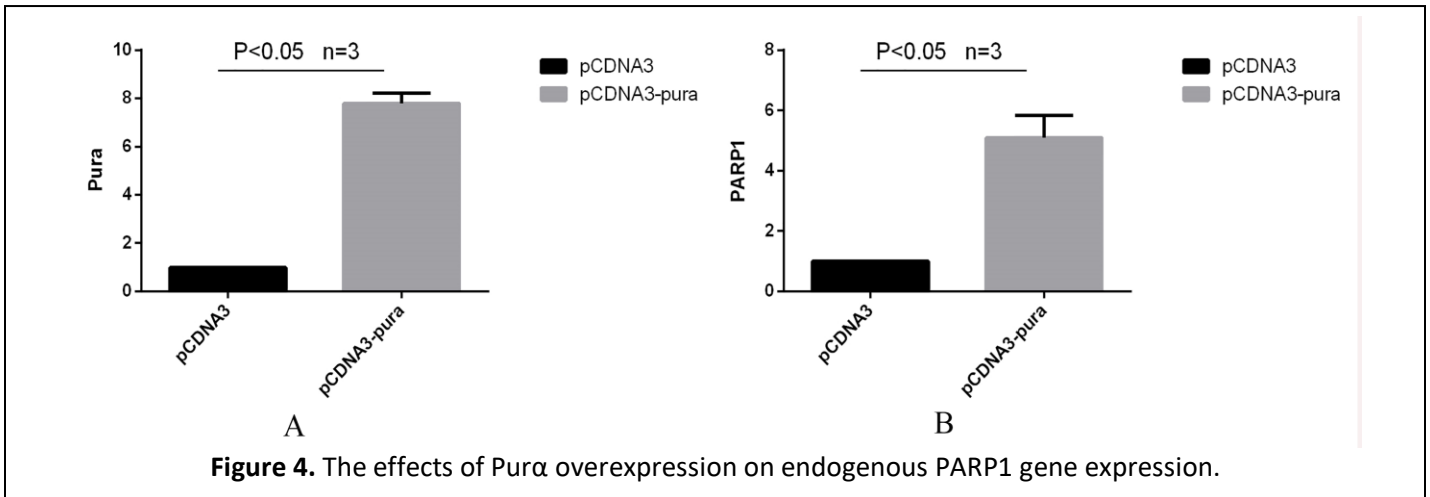
PARP1 gene expression at the transcriptional level, U87MG cells were transfected with the eukaryotic expression plasmid, pCDNA3.0-Purα, and the control plasmid, pCDNA3.0. After the transfection (48 hours), the cellular total RNA was extracted and applied to the qPCR assay. The RNA contents of Purα and PARP1 were evaluated with qPCR to confirm that the transfected Purα had been overexpressed and to investigate the effects of Purα on PARP1 gene expression. The results of the qPCR demonstrated that compared with control group (pCDNA3) the content of PARP1 RNA in the Purα group increased remarkably. The results indicated that Purα significantly upregulated PARP1 gene expression, which is consistent with the results of the luciferase assay, which indicated that Purα promotes the expression of PRAP1 (Fig 4).

Western blot assays
To investigate the effects of Purα on regulation of PARP1 gene expression at the translational level, U87MG cells were transfected with the eukaryotic expression plasmid of pCDNA3.0-Purα and the control plasmid, pCDNA3.0. The total cellular proteins were extracted and Western blot assays were performed. The results illustrated that

Figure 3. The effects of Purα on PARP1 promoter activity when cotransfected into U87MG cells.

Figure 4. The effects of Purα overexpression on endogenous PARP1 gene expression.

Figure 5. Western blotting analysis assessed the effects of Purα on PARP1 gene expression. (A) Western blotting assay to determine the effects of Purα overexpression on PARP1 gene expression. (B) (C) The statistical analysis results of Western blotting.

Figure 6. The effects of Purα on PARP1 gene expression and alleviated the DNA damage. (A) The results of Western blotting show the effects of Purα on PARP1 gene expression within DNA damage; (B) (C) The statistical analysis results of Western blotting.

Purα had a remarkably positive regulatory effect on the expression of PARP1 (Fig 5).

Figure 7. The results of purification of GST and GST-Purα proteins.
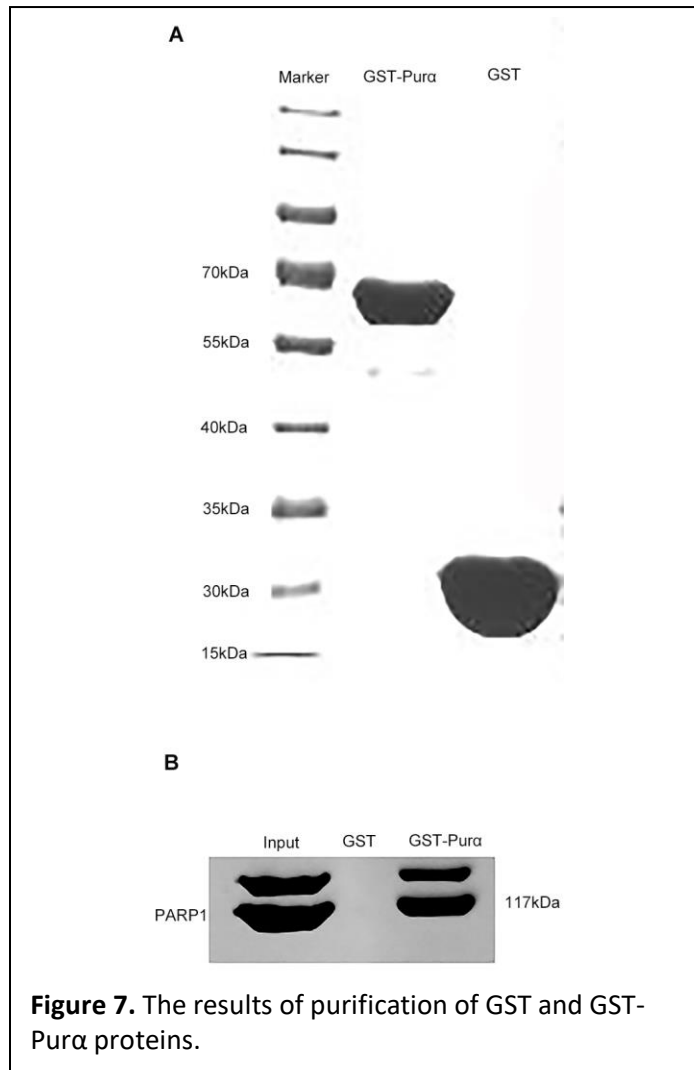
In order to explore the interaction between Purα and PARP1 in the context of DNA damage, hydroxyurea (HU) was used to build a model of DNA damage and then the expression of Purα and PARP1 were evaluated. To observe the dynamic changes in the DNA damage induced by HU, the content of γH2AX at different time points was checked (0 h, 3 h, and 6 h after the HU treatment). The results indicated that after the HU treatment, DNA damage occurred and γH2AX content increased dramatically at the 0 h time point. As time passed, the content of γH2AX decreased. That meant that the repair system inside the cells had been awakened and the cells started to repair the damage. By the 6 h time point after the HU treatment, the content of γH2AX returned to less than at the time of the HU treatment. Compared with the Purα overexpression group, the expression of PARP1 was much lower in the control group. On the contrary, the contents of γH2AX was much higher in the control group than in the Purα overexpression group at each time point. Purα had a higher expression level in the overexpression group compared with the control group (Fig 6). The results demonstrated that HU could induce DNA damage and γH2AX is a very sensitive early DNA damage signal protein. PARP1 was upregulated with Purα overexpression. With greater expression of PARP1 and higher levels of Purα expression, the content of γH2AX decreased, which indicated that Purα could up regulate the PARP1 gene expression and as a result, it alleviated the HU-induced DNA damage.

Figure 8. The interaction between Purα and PARP1 was assessed by pull down assay.
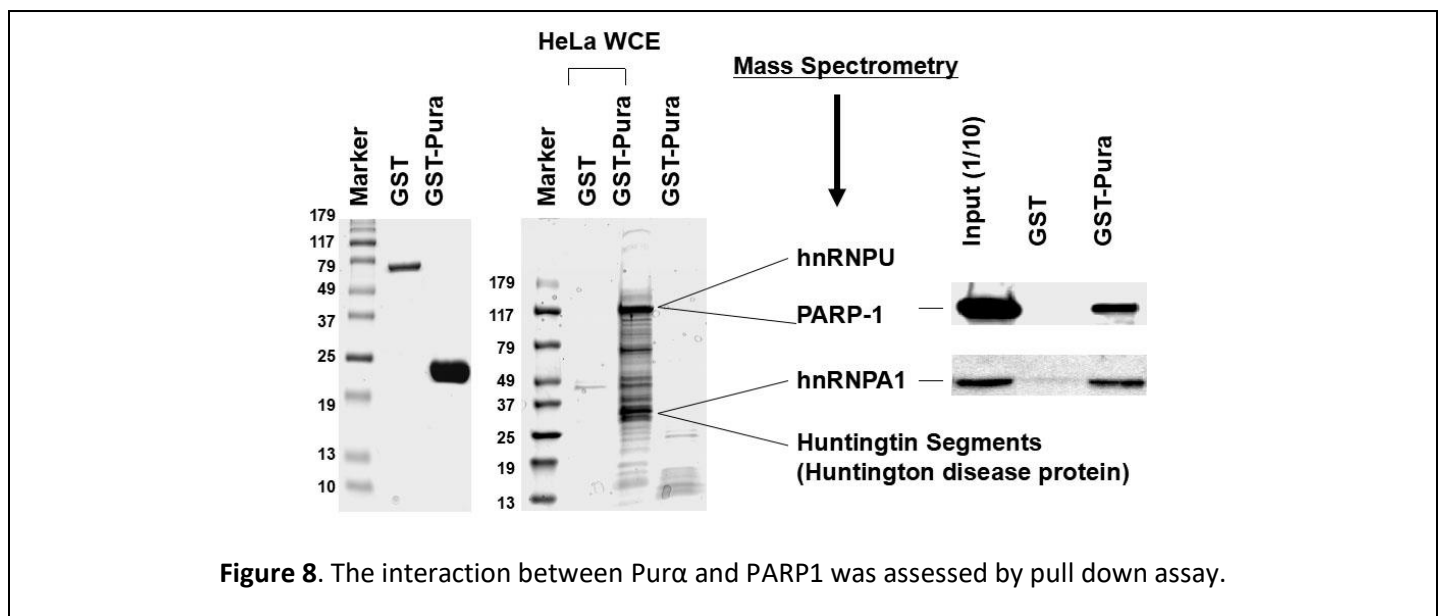
Pull down assay

The GST fusion protein Purα and GST control were purified from *in vitro* induced prokaryote recombined bacteria. The protein was purified with glutathione sepharose beads and used as an immobile phase. The purified GST and GST-Purα were applied to polyacrylamide gel electrophoresis (PAGE) to check the purity of the proteins. After PAGE, the gel was stained with Coomassie Brilliant Blue Staining solution and then destained. The results demonstrated that purified GST was located at 26 kDa, while the fusion GST-Purα was located at 63 kDa compared with the 37 kDa for Purα (Fig 7). The sepharose beads linked protein was used to pull down HeLa cell whole extracts in order to identify the physical interaction with PARP1. The results (Fig 8) illustrated that Purα could pull down PARP1 protein from HeLa whole cell extracts. This indicated the presence of physical interactions between PARP1 and Purα.

DISCUSSION & CONCLUSION Accumulating evidence suggests that PARP is a form of nucleic enzyme that is located in the nucleus of eukaryotic cells and catalyzes polyADP-ribose polymerization, and thus plays a crucial role in repairing and recognizing DNA damage, chromosome remodeling, the formation of the mitotic apparatus, and cell death. Over-activation of PARP is involved in many diseases. The main isoform, PARP1, is critical for DNA damage and repair functions. This function is also related to oncogenesis and chemotherapy resistance. Historical studies have identified 18 members of the PARP family, of which the most important and abundant is PARP1. PARP1 repairs SSBs via the BER pathway. Damaged DNA activates PARP, which cleaves NAD^+ into nicotine and ADP-ribose. The ADP-ribose then forms polyADP-ribose (PAR), combining with the receptor that contains PARP1, histones, and other proteins for DNA repair. Dissociation from PARP1 occurs at the DNA breaks when PARP1 is modified by PAR, which adheres to the breaks instead. The polymer has many negative charges, and thereby recruits major proteins in the BER-SSBs pathway, such as XRCC1. Other proteins that contribute to the spatial structural formation of chromosomes and DNA repair and replication are non-covalently bound to PAR, as well. These recruitment effects collaborate with noncovalent binding to exert DNA repair. Recent work has suggested that PARP is involved in more DNA damage and repair functions, such as recruiting MRE11 and ATM, inhibiting E2F4 and P130 compounds to influence the expression of BRCA1 and RAD51,[22] and interacting with protein kinase complexes for the repair of DNA DSBs. Studies have shown that cells treated with alkylating agents, PARP1 inhibitors, or RNAi inhibited the expression of PARP1 and resulted in different consequences of DNA damage and repair. PAR can be catalyzed and synthesized by activated PARP1, which performs protein modification functions after translation in the cells.

A recent study confirmed the function of Purα in cell survival and differentiation in transgenic mice. Neuron numbers were decreased significantly in the hippocampus and cerebellum of young mice, which indicated that Purα played a crucial role in the development of neurons. The Purα (-/-) cell line showed chromosomal abnormalities when treated with genotoxic agents, which suggested that Purα was vital to DNA damage and repair in neurodegenerative diseases. Additionally, another study demonstrated that Purα executed surveillance in DSBs that was caused by replication fork halt. The polyribosome and hnRNP joined Purα in forming a complex that was enriched in the cytoplasm of neuronal cells, especially in the branch point of synapses. Purα also contributed to the stability of the genome and was exclusively expressed in specific tissues. A previous study confirmed that Purα mediated a specific mechanism of transport and translation, while others have found that a mutation in Purα can cause profound neonatal hypotonia, seizures, and encephalopathy in 5q31.3 microdeletion syndrome.[23] Hunt *et al.* revealed by whole exome sequencing in family trios that de novo mutations in Purα led to neurocognitive disorders, severe neurodevelopmental delays, and learning disabilities.[24]

Our study is the first that illustrated that Purα upregulated PARP1 gene expression. Our results demonstrated that when Purα was overexpressed, the PARP1 promoter activities were activated. Our qPCR and western blot results also confirmed that Purα could positively regulate PARP1 gene expression both at transcriptional and translational levels. Our experiment also found that when the Purα gene was knocked out, the PARP1 expression level in mouse embryo fibroblast cells (Purα-/- MEFs) was much lower than in their wild type cells. We also found that re-transfected Purα genes in the Purα-/- MEFs, can successfully reverse PARP1 gene expression (data not show). These results confirmed that Purα plays an important role in PARP1 gene expression. Although the detailed mechanisms for this up-regulation on PARP1 gene expression are unclear, as a transcriptional factor, Purα could be an important regulator of gene expression. The regulatory functions exhibited great variances and complexity. For some genes, Purα exerted a positive regulatory effect, and acted as a transcriptional activator, such as the neuron-specific FE65 protein and PDGF-A. For other genes, Purα had a negative effect on transcription,

such as with amyloid precursor protein. Purα also negatively regulated its own promoter activities.[21]

Using the pull-down assay, we found that some physical interactions existed between Purα and PARP1. No similar reports have been previously published, to our knowledge. This physical interaction might explain how Purα and PARP1 together exert the up-regulatory effects on the PARP1 gene expression, but a detailed view of the mechanism requires further investigation. Surprisingly, in the second band of the pull-down assay (Fig 8), we found heterogeneous nuclear ribonucleoprotein A1 and Huntingtin C-terminal segment (Huntington disease protein). The interaction of Purα with hnRNP A1 has been confirmed by western blot and immunoprecipitation (data not shown).

PARP1 is involved in the response to DNA damage. Our results showed that Purα is involved in DNA repair, probably by preventing and repairing DNA DSB formation at stalled replication forks. Purα is also associated with PARP1 to maintain poly(ADP-ribose) (PAR) homeostasis in the central nervous system. We also noted the effects of PARP1 on DNA damage and repair. The change in PARP1 after DNA damage was consistent with the previous work that demonstrated that HU was unable to inhibit the transcription of PARP1. Reports claim that in the early stages of cell damage, extracellular signals activate PARP1 through the MAPK pathway, a pathway involved in immune responses and inflammation. For instance, ROS activates PARP1 through the MAPK pathway to influence apoptosis.

The effects of Purα and PARP1 on the development of the neuronal system have previously been described. Those studies focused on the protective effects of Purα on neurons, the contribution of PARP1 to SSBs, and the PARP1 inhibitor in cancer treatment. Few studies concentrated on the effects of Purα on the expression of PARP1. Here, we demonstrated that Purα regulates the function of PARP1, providing multiple lines of evidence for the influence of Purα and the interaction between the two proteins. This relationship implies a possible route for anticancer drug development. Furthermore, we plan to perform immunofluorescence techniques to investigate the distribution of Purα and PARP1 intracellularly in the damage model, and we plan to employ co-immunoprecipitation to study the status of these two proteins intracellularly. Next, we plan to construct various deletion mutants and nucleic acid-protein binding experiments, such as EMSA, to examine the binding point of Purα and the promoter of PARP1.

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