Pathology - Research and Practice 215 (2019) 152567

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

PDRG1 gene silencing contributes to inhibit the growth and induce apoptosis of gastric cancer cells

Ya-Jing Zhang, Jing-Quan Li, Hua-Zhi Li, Hui Song, Chang-Sheng Wei, Shu-Qi Zhang*

Department of General Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing, China

ARTICLE INFO

Keywords:
PDRG1
Gastric cancer
ATM/p53

ABSTRACT

Objective: This paper aims to provide some experimental basis for unveiling the role of PDRG1 (P53 And DNA Damage-Regulated Gene 1) gene silencing in the growth and development of gastric cancer.

Methods: PDRG1 levels in gastric cancer tissues and cell lines were measured by Western blotting. Then, gastric cancer BGC-823 cells, divided into Control, PDRG1 siRNA, NC siRNA and PDRG1 siRNA + KU55933 (ATM inhibitor) groups, were used to conduct a series of *in vitro* experiments including MTT, Flow cytometry, Wound-healing and Transwell assays. Expression of PDRG1 and ATM/p53 pathway-related proteins were determined by Western blot. Eventually, experiment *in vivo* was carried out to verify the control of PDRG1 on gastric cancer cells after establishing the tumor xenograft model in nude mice.

Results: PDRG1 was significantly elevated in gastric cancer tissues and was associated with lower cell differentiation degree, more severe lymph node metastasis and higher tumor stage of gastric cancer patients. The growth of BGC-823 cells were significantly retarded and the cell apoptosis was increased in the PDRG1 siRNA group; besides, cell cycle was arrested in G2/M phase, and the expressions of p-ATM, p53, p21, p-cdc2 and cleaved caspase-3 were up-regulated with the reduced PDRG1. However, KU55933 could reverse the anti-tumor effect of PDRG1 siRNA on BGC-823 cells. The *in-vivo* experiment confirmed PDRG1 siRNA can inhibit tumor xenograft growth in nude mice.

Conclusion: Specific PDRG1 gene silencing may inhibit the growth and metastasis of gastric cancer cells through the activation of ATM/p53 pathway.

1. Introduction

Gastric cancer, one of the most prevalent malignancies, has the fourth incidence among all cancers worldwide, which only next to lung cancer, breast cancer and colorectal cancer, making itself the second leading cause of cancer-induced human death [19,29]. According to relevant studies, the early clinical symptoms and signs of gastric cancer are not specific and prone to be neglected, thus most patients diagnosed with gastric cancer are in the middle or late/advanced stage and miss the opportunity of radical operation, resulting in the very low 5-year survival rate of only approximately 25%–30% [7,34]. When it comes to the pathogenesis of cancers, DNA damage is the major factor to be brought up. As proved by many researchers, the occurrence and progression of many different cancers were largely caused by the failure of repair of DNA damage, the abnormal accumulation of gene mutations and the genetic instability [18,32].

As a gene shorts for the regulation of p53 and DNA damage, PDRG1 has been discovered for many decades and it was reported to have a close bearing on various cellular physiological activities, including cell proliferation, growth, apoptosis, cell cycle regulation, and DNA damage repair [17]. Recently, the role of PDRG1 has been described as an oncogenic molecule, for instance, in colorectal cancer tissues and gastric cancer tissues, PDRG1 was observed to be highly expressed when compared with the corresponding normal tissues, whereas the knockout of PDRG1 in human colon cancer cells could affect the cancer cell growth [8]. As analyzed by Tao Z et al., PDRG1 could affect the growth and radio-sensitivity of lung cancer cells by mediating the ATM-P53 signaling pathway [23]. Importantly, *ATM* gene has been demonstrated by previous evidence to be able to sense DNA damage, transmit DNA damage signals to downstream target genes, and then initiate stress systems, to induce cell cycle arrest, cell repair and even cell apoptosis [14,22]. As the downstream substrate of ATM, p53 can sense the phosphorylation signal of ATM and cause cell cycle arrest or cell apoptosis [28]. In addition, it has been proved that

* Corresponding author at: Department of General Surgery, Beijing Anzhen Hospital, Capital Medical University, No. 2, Anzhen Road, Chaoyang District, Beijing 100029, China.

E-mail address: zhangzhsq0311@126.com (S.-Q. Zhang).

https://doi.org/10.1016/j.prp.2019.152567
Received 13 March 2019; Received in revised form 10 July 2019; Accepted 26 July 2019

ATM-P53 signaling pathway has a significant impact on the growth, apoptosis and cell cycle distribution of gastric cancer cells [12,21]. However, though much progress has been made in explaining the pathogenesis of gastric cancer, it is still limitedly stated about the impact of PDRG1 gene silencing on gastric cancer. Here, we aimed at assessing the role of PDRG1 and the ATM-P53 signaling pathway in the invasiveness, proliferation and metastasis of gastric cancer cells.

Therefore, in order to further study the effect of PDRG1 expression on the growth and progression of gastric cancer cells via ATM-P53 pathway, experiments *in vivo* and *in vitro* were performed, aiming to provide some guidance on the further exploration of appropriate prevention and treatment of gastric cancer in human.

2. Materials and methods

2.1. Ethics statement

The study was carried out with approval from the Ethics Committee of Beijing Anzhen Hospital. The procedures of all experiments complied with the protocols in the Helsinki Declaration. Besides, the informed consent was signed by all patients or their families prior to the collection of tissues samples.

2.2. Research subjects

From January 2016 to January 2018, we collected 68 gastric cancer tissue specimens from patients who underwent radical gastrectomy for gastric cancer in the Department of Gastrointestinal Surgery at our hospital. All specimens were histopathologically confirmed as gastric cancer. The average age of patients was 51.79 ± 12.25 years including 41 males and 27 females. Among these patients, 27 cases were highly differentiated and 41 cases poorly differentiated; 37 cases were with lymph node metastasis and 31 cases without metastasis; and 29 cases were in stage I—II and 39 cases in stage III-IV. In the meantime, adjacent normal tissues about 5 cm to the tumor tissues were collected as the Control group. All patients had no history of other digestive system tumors or any other tumors and they received no chemotherapy or radiotherapy before the operation.

2.3. Cell culture

Human gastric cancer cell lines (BGC-823, MGC-803, SGC-7901, AGS and MKN-45) and normal gastric epithelial cells (GES-1) were all purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were grown in an incubator (Thermo, USA) with the constant temperature of 37 °C and 5% CO_2 and were maintained in RPMI1640 medium (31800-022, Hyclon, VA, USA) supplemented with 10% inactivated fetal bovine serum (FBS, 16000-044, Gibco, Carlsbad, CA, USA) and 100 units/ml penicillin-streptomycin (15140122, Gibco, USA).

2.4. Cell grouping and transfection

BGC-823 cells were selected for subsequent experiments, with the following four experimental groups: Control, PDRG1 siRNA, NC siRNA and PDRG1 siRNA + KU55933. BGC-823 cells in the Control group without any treatment. Meanwhile, Cells in the PDRG1 siRNA group and NC siRNA group were transfected with PDRG1 siRNA and NC siRNA respectively, while those in the PDRG1 siRNA + KU55933 group were transfected with PDRG1 siRNA and cultured in medium containing KU55933 (ATM inhibitor, 10 μmol/L, Sigma-Aldrich) [11]. Sangon Biotech (Shanghai) Co., Ltd helped to synthesize the sequences of PDRG1 siRNA and Negative control siRNA for our experiment. The cells were seeded into 24-well plates and allowed to grow to confluence and routinely passaged till the cell confluence reached 70%–80%. Next, the transfection mixture was prepared by following the instructions of the Lipofectamine 2000 kit (Invitrogen) in the next day. PDRG1 siRNA and NC siRNA sequences were diluted by 250 μl serum-free medium (final concentration 50 nM), and another 250 μl serum-free medium was used to dilute 5 μl lipofectamin 2000, before 5 min of incubation at room temperature. The two reaction solutions were then mixed and kept at room temperature for 20 min and transferred to the holes for another 6 h of incubation at 37 °C and 5%CO_2 with saturated humidity. Ultimately, the culture medium was replaced with RPMI 1640 medium containing 10% FBS to boost the nutrition.

2.5. MTT assay

Transfected cells were washed with PBS twice and digested with 0.25% trypsin to make the single-cell suspension for later use. After cell counting, cells were inoculated into 96-well plates by 3×10^3-6×10^3 cells/well, with the volume of each hole being 200 μL and 6 replicates being set. After 48 h of incubation, 20 μL of MTT solution (5 mg/ml, A2776-1 g, Shanghai Shifeng Biological Technology Co., Ltd., China) was added into cells which were to be cultured for 4 h. For the next step, the culture medium in each well was removed and 150 μL of DMSO was added for 10 min of gentle shaking. At the 24th, 48th, and 72nd h of cell culture, an Enzyme-Linked ImmunoSorbent Assay (ELISA) was performed to detect the absorbance (OD value) of each well at the wavelength of 490 nm.

2.6. Flow cytometry for evaluation of cell apoptosis and cell cycle

To evaluate the degree of cell apoptosis, cells were digested with trypsin, centrifuged and collected, and washed with cold PBS, before the suspension with calcium-containing PBS to obtain single-cell suspension with the density of 1×10^6/ml. Next, 100 μl single-cell suspension was taken at room temperature and added with 10 mg/ml propidium iodide (PI, P4170, Sigma-Aldrich) and 10 mg/ml Nase A. After 30 min of incubation at 4 °C, 400 μl staining buffer was added immediately before the detection and analysis with a flow cytometer (BD Bioscience). Each time 10^4 cells were obtained for data analysis by the software Cell Quest. Repeated experiment was performed for another two times independently to obtain the mean value. Cells in the logarithmic growth phase were harvested and seeded into 6-well plates at a final density of 5×10^5 cells/well to evaluate the proportion of cells in different cell cycle phases. The pre-cooled 70% ethanol was added for 12 h of placement at 4 °C, followed by washing cells thrice with PBS and the addition of l ml PI dye for 20 min of light-shielding reaction at 4 °C. In the end, the number of cells in different phases of the cell cycle was counted by using the flow cytometer.

2.7. Wound-healing assay

Cells were seeded at 1×10^5 cells/well in 6-well plate and incubated at 37 °C with 5% CO_2. When cell confluence reached 90%, cells were starved in serum-free medium for 24 h and then a line was gently drawn across the well with a sterilized pipette tip. It is important to ensure the same scratch width as possible. Next, PBS was used to wash away floating cells and serum-free medium was added for 0.5–1 hours of incubation to restore the starved cells. When cells were recovered from starvation, it was recorded as 0 h. Cells were observed and their pictures were taken under an Olympus inverted microscope at 0 h and 48 h. Migration was quantified by the number of cells migrated to the scratch area. Each number represents the average count of cells in three independently scratch assays.

2.8. Transwell invasion assay

The matrix gel used in this study, Matrigel (40111ES08, Shanghai Yeasen Biology Co. Ltd., China), was dissolved at 4 °C overnight and then diluted in serum-free DMEM medium (1: 3) before evenly covering

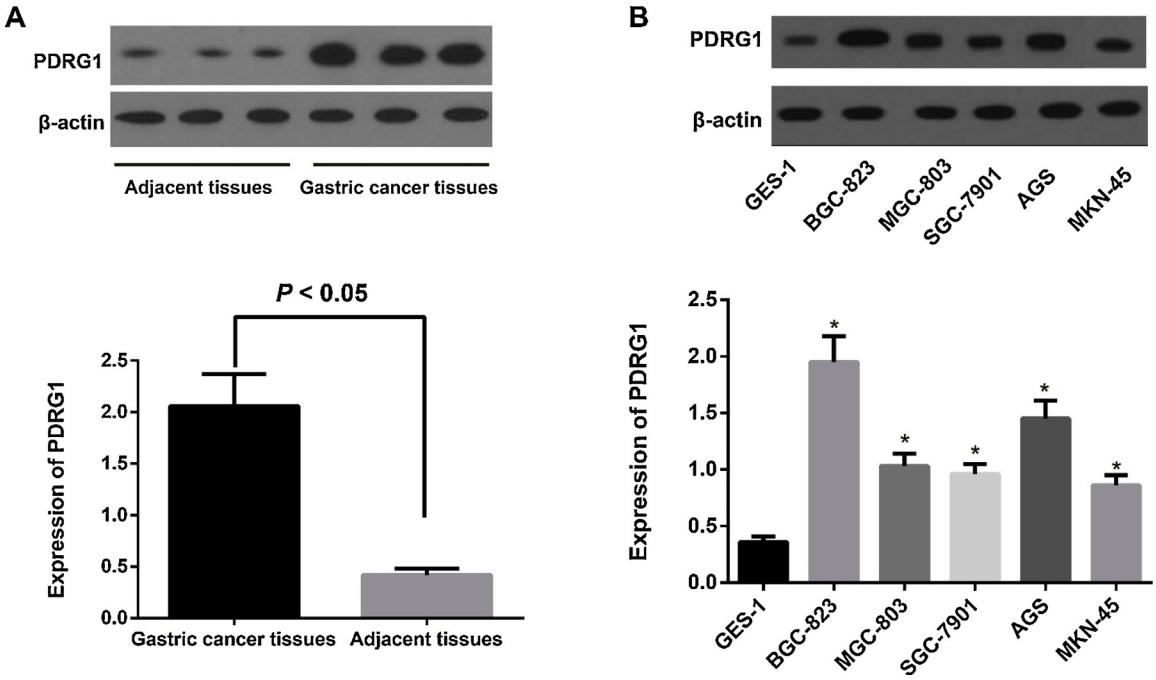

Fig. 1. Comparison of the expression of PDRG1 in gastric cancer tissues and cell lines.
Note: A, Western blot detecting the expression of PDRG1 in gastric cancer tissues and adjacent normal tissues; B, Comparison of the PDRG1 expression between gastric cancer cell lines (BGC-823, MGC-803, SGC-7901, AGS and MKN-45 cells) and normal gastric epithelial cells GES-1; *, $P < 0.05$ compared with GES-1 cells.

the upper room of Transwell chamber. A single-cell suspension were collected and added into the upper chamber of the Transwell system to achieve cell inoculation at 48 h after the transfection. In the meantime, the lower chamber was loaded with 0.5 ml DMEM medium added with 10% FBS. The Transwell chamber system was put inside an incubator for 48 h of incubation (37 °C, 5% CO_2). Subsequently, Transwell system was taken out and the cells on the upper chamber were removed. Cells penetrating the upper chamber were fixed in ethanol (5%, 15–20 min) and stained with crystal violet for 10 min. To observe cell invasion, five high-power visual fields under an inverted microscope were randomly chosen for cell counting and the average value of each cell sample was obtained accordingly. Eventually, the number of cells invading through the Matrigel of lower chamber was counted and each experiment was repeated three times independently.

2.9. Western blot

Transfected cells were collected after incubation for 48 h and proteins were extracted, and the proteins concentration was determined by using the BCA method (Boster Biological Technology Co. Ltd.). Loading buffer was added into the extracted proteins for 10 min of heating at 95 °C and 30 μg sample solution was added in each hole. Next, electrophoresis was performed with 10% polyacrylamide gel (Boster Biological Technology Co. Ltd.) to separate proteins. The isolated proteins were transferred to the PVDF membrane before 1 h of blocking in 5% BSA at room temperature. Afterwards, primary antibodies were added into the proteins for overnight reaction at 4 °C, including PDRG1 (ab131219, abcam), p-ATM (ab79891, abcam), p53 (ab32389, abcam), p21 (ab188224, abcam), p-cdc2 (77055, Cell Signaling Technology Inc., Beverly, MA, USA), cleaved caspase-3 (ab49822, abcam), and β-actin (ab8226, abcam). In the next step, the PVDF membrane with proteins was taken and washed with TBST 3 times (5 min each time) and corresponding secondary antibodies were incubated for 1 h. At last, the membrane was washed again with TBST for three times, 5 min each time, and chemiluminescent reagent was applied for color development. With β-actin being the internal reference gene, the software Image J was chosen for the analysis of the gray value of the target bands. The experiment was carried out three times independently.

2.10. Establishment of tumor xenograft model in nude mice

There were 24 BALB/c-nu nude mice (SPF grade) used as laboratory animals in this study and the mice were 3–4 weeks old and weighing 15–18 g, all provided by the Institute of Laboratory Animal Sciences, CAMS&PUMC. During the experiment, cells of Control, PDRG1 siRNA, and NC siRNA groups were collected separately at the logarithmic growth phase and serum-free RPMI-1640 medium was used to prepare single-cell suspensions (3×10^{10} cell/L) necessary for the subsequent experiment. Next, 0.2 ml single cell suspension was inoculated subcutaneously on the back of 24 nude mice, 8 in each group. After that, the length (a) and width (b) of the tumors were measured with vernier calipers once every 3 days for 5 weeks in a row, and the tumor growth curve was drawn based on the data recorded. The volume of tumors was measured using the following formula: $V = (ab^2)/2$. After the performance of all experiments, the nude mice were sacrificed by dislocating the neck and tumor weight was measured.

2.11. Statistical methods

Statistical software SPSS 21.0 was used for quantitative data analysis. Measurement data were analyzed statistically and expressed as mean ± standard deviation ($\bar{x} \pm s$). The comparison between the two groups of measurement data complying with normal distribution was conducted by Studen's t-test, while the comparison among multiple groups was analyzed by One-Way ANOVA and Tukey's HSD Post Hoc test. The enumeration data were expressed as percentage and rate and tested by using Chi-square test. $P < 0.05$ was regarded as significant difference.

3. Results

3.1. Expression of PDRG1 in gastric cancer tissues and cells

As a result, the expression of PDRG1 was significantly higher in

Table 1
Relationship between PDRG1 expression and clinicopathological features of patients with gastric cancer.

Clinicopathological features	N	PDRG1 expression	*P*
Age			*0.785*
< 55	37	2.03 ± 0.29	
≥ 55	31	2.05 ± 0.31	
Gender			*0.401*
Male	41	2.04 ± 0.29	
Female	27	2.10 ± 0.28	
Tumor size			*0.579*
≤ 5 cm	30	2.06 ± 0.31	
> 5 cm	38	2.02 ± 0.28	
Differentiation degree			*< 0.001*
Moderate/Well	27	1.76 ± 0.16	
Poor	41	2.26 ± 0.21	
TNM stage			
I-II	29	1.78 ± 0.17	*< 0.001*
III-IV	39	2.27 ± 0.21	
Lymphatic metastasis			
Yes	37	2.29 ± 0.20	*< 0.001*
No	31	1.79 ± 0.17	

gastric cancer tissues than in adjacent tissues, as detected by Western blot ($P < 0.05$, Fig. 1A). Moreover, in different gastric cancer cells shown in Fig. 1B, the gastric cancer cells (BGC-823, MGC-803, SGC-7901, AGS and MKN-45) had significantly up-regulated expression of PDRG1 compared to the normal gastric epithelial cells GES-1, among which BGC-823 cells had the highest PDRG1 level (all $P < 0.05$). After analyzing the relationship of PDRG1 expression with the clinicopathological features of patients for gastric cancer, we found PDRG1 expression was not bound up with the gender, age and tumor size of patients, but was significantly associated with the lower cell differentiation degree, more severe lymph node metastasis and higher tumor grade of gastric cancer patients (all $P < 0.05$, Table 1).

3.2. The effects of PDRG1 silencing on the proliferation of gastric cancer cells

MTT assay was used to detect the proliferative ability of gastric cancer cells in each transfection group (Fig. 2). Obviously, compared with cells in the Control group, BGC-823 cells in the NC siRNA group demonstrated no observable difference ($P > 0.05$), while those in the PDRG1 siRNA group were significantly reduced regarding cell proliferative ability ($P < 0.05$). Besides, cell proliferation was stronger in the PDRG1 siRNA + KU55933 group than in the PDRG1 siRNA group ($P < 0.05$).

3.3. The effect of PDRG1 gene silencing on the apoptosis and cell cycle of gastric cancer cells

As seen in Fig. 3, the PDRG siRNA group formed an obvious uptrend regarding the cell apoptosis rate, an appreciable decrease in the proportion of cells in the S phase, and reflected a cell cycle arrest in the G2/M phase as compared with the Control group (all $P < 0.05$). However, the NC siRNA group didn't show observable difference regarding those indexes in comparison with the Control group (all $P > 0.05$). However, the cell apoptosis rate and the cell proportion in the G2/M phase were lower with a higher proportion of S phase in the PDRG1 siRNA + KU55933 group than that in the PDRG siRNA group (all $P < 0.05$), which suggested that PDRG1 siRNA could cause cell cycle arrest of gastric cancer cells at the G2/M phase.

3.4. PDRG1 gene silencing inhibits the migration and invasiveness of gastric cancer cells

Compared to Control group, NC siRNA group didn't have any significant difference regarding the migration and invasiveness of gastric cancer cell line BGC-823 cells (all $P > 0.05$), while PDRG1 siRNA group was apparently decreased in the number of migrated cells and invasive cells (all $P < 0.05$). Meanwhile, PDRG1 siRNA + KU55933 group was obviously higher in the number of both migrated and invasive gastric cancer cells than the PDRG siRNA group (all $P < 0.05$, Fig. 4).

3.5. Expression levels of PDRG1 and ATM/p53 pathway-related proteins

To detect the expression levels of PDRG1 and ATM/p53 pathway-related proteins in gastric cancer BGC-823 cells after transfection with siRNA, we performed Western blot according to standard procedures (Fig. 5). As a result, BGC-823 cells in the PDRG1 siRNA group were significantly down-regulated in the expression of PDRG1, but statistically up-regulated in the protein expression of p-ATM, p53, p21, p-cdc2 and cleaved caspase-3, as compared to those in the Control group (all $P < 0.05$). Meanwhile, compared with PDRG siRNA group, PDRG1 siRNA + KU55933 group did not differ statistically in the PDRG1 expression level in gastric cancer cells, but was significantly reduced in the protein expression levels of p-ATM, p53, p21, p-cdc2 and cleaved caspase-3 (all $P < 0.05$).

3.6. Impact of silencing PDRG1 on the growth of subcutaneous tumor xenograft in nude mice

One week after the transplantation of tumor cells, all nude mice had tumor xenografts growing in their body, indicating the tumorigenic rate of 100%. Compared with mice in the Control group, those in the PDRG1

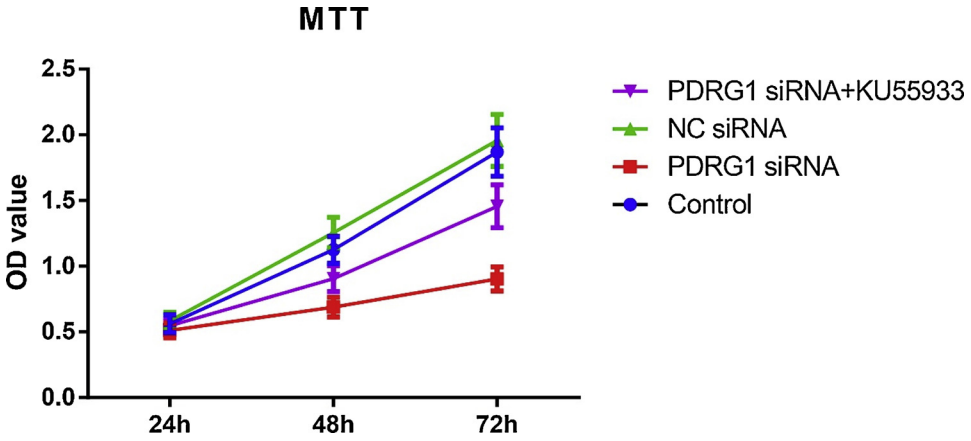

Fig. 2. MTT assay was used for analyzing the effect of PDRG1 gene silencing on the gastric cancer BGC-823 cells proliferation.

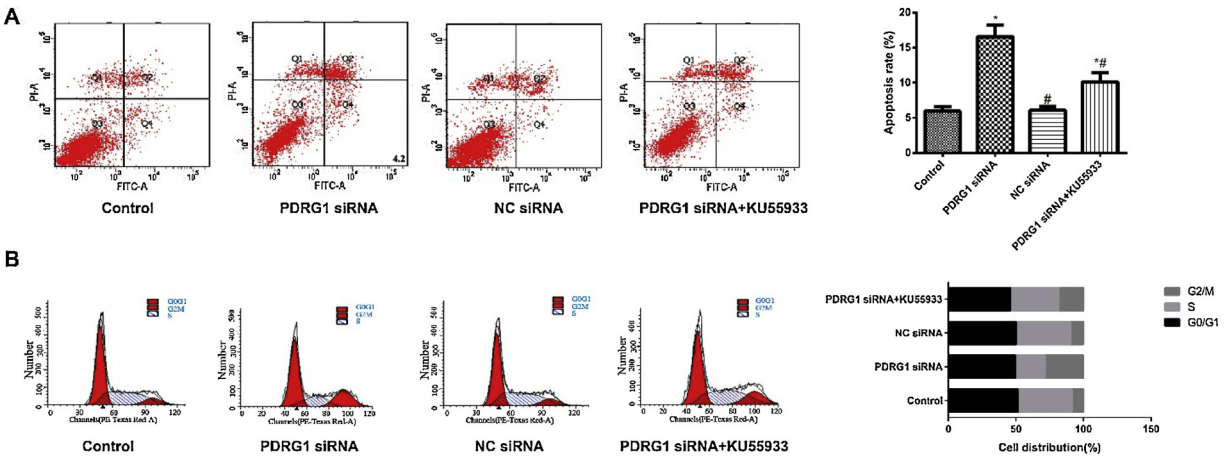

Fig. 3. Impact of PDRG1 on the apoptosis and cell cycle of gastric cancer BGC-823 cells.
Note: A–B, Effect of silencing PDRG1 on the apoptosis (A) and cell cycle (B) of BGC-823 cells measured by flow cytometry; *, $P < 0.05$ compared with Control group; #, $P < 0.05$ compared with PDRG siRNA group;

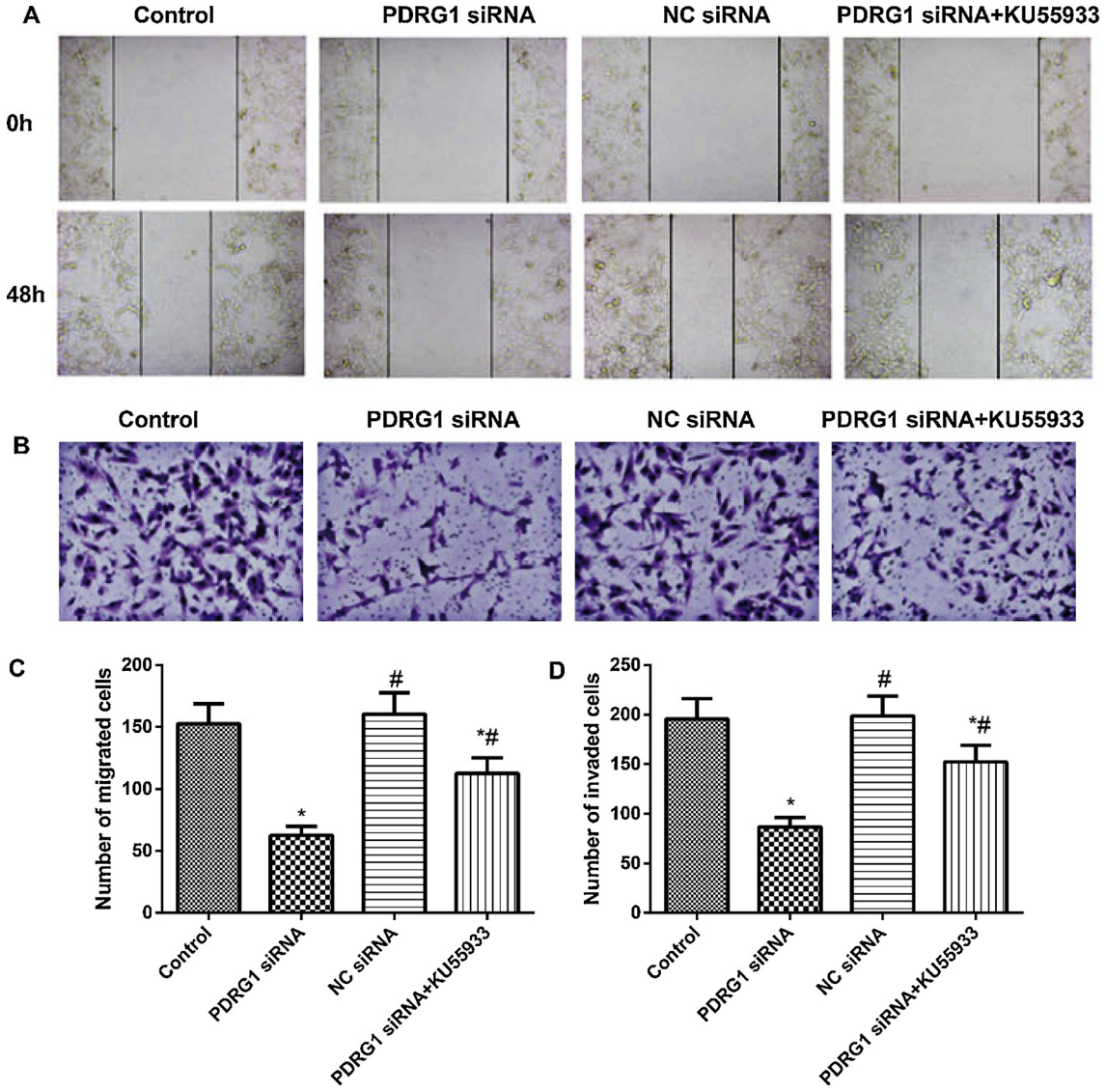

Fig. 4. The effect of PDRG1 gene silencing on the migration and invasiveness of gastric cancer BGC-823 cells.
Note: **A**, Effect of silencing PDRG1 on the migration ability of BGC-823 cells evaluated by Wound-healing assay; B, Effect of silencing PDRG1 on the invasive ability of BGC-823 cells measured by Transwell invasive assay; C–D, Comparing the numbers of migrated cells (C) and invasive cells (D) among different transfection groups; *, $P < 0.05$ compared with Control group; #, $P < 0.05$ compared with PDRG siRNA group.

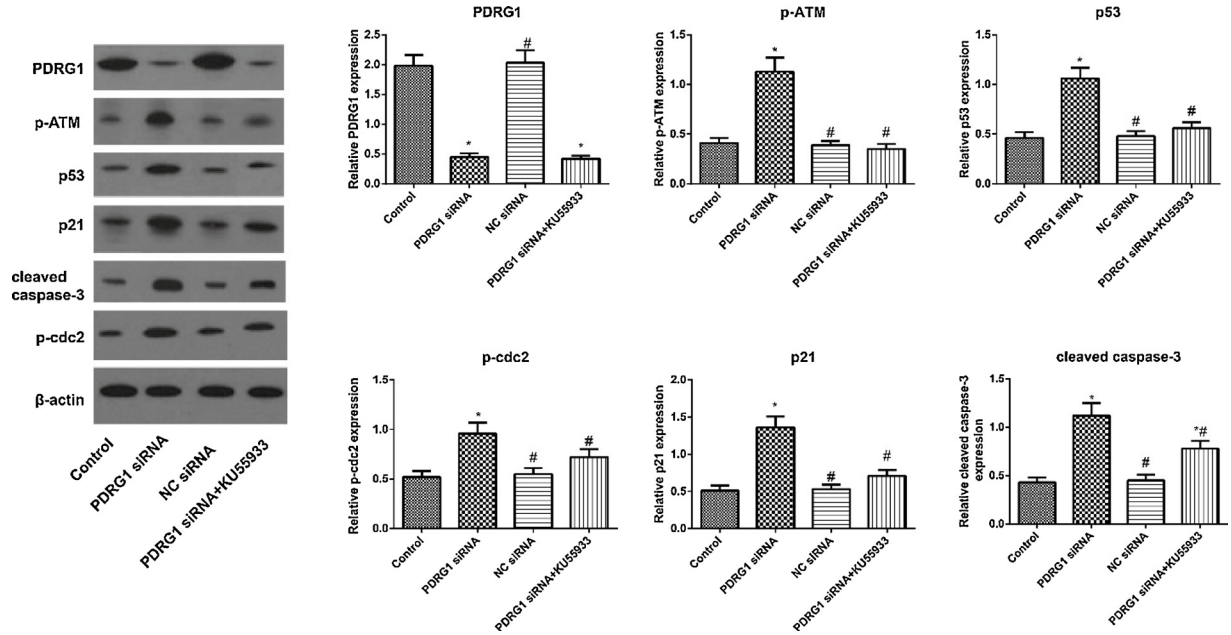

Fig. 5. Expression of PDRG1 and ATM/p53 pathway-related proteins in gastric cancer BGC-823 cells detected by Western blot.
Note: *, $P < 0.05$ compared with Control group; #, $P < 0.05$ compared with PDRG siRNA group.

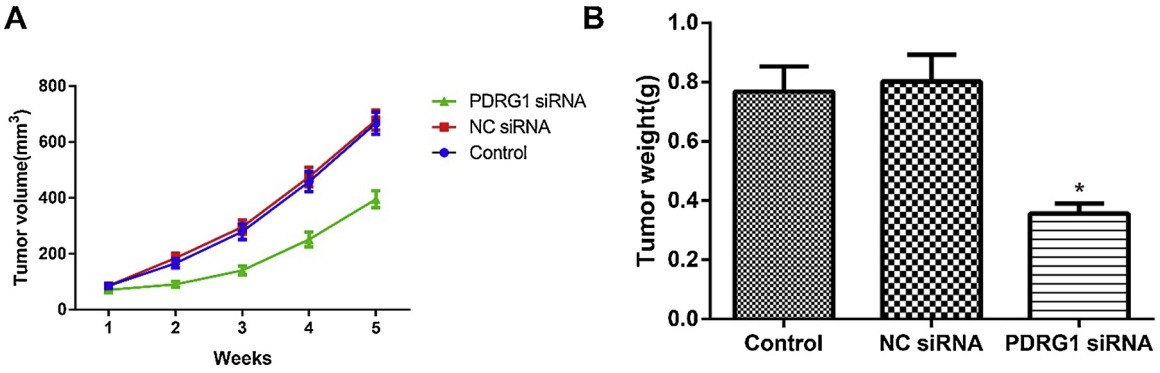

Fig. 6. Effects of silencing PDRG1 on the growth of subcutaneous tumor xenograft in nude mice.
Note: A, Growth curve of subcutaneous tumors in nude mice of each group; B, Comparison of tumor weight among different groups; *, $P < 0.05$ compared with Control group.

siRNA group were apparently slowed down in tumor growth rate and appreciably reduced in tumor weight (both $P < 0.05$), while mice in the NC siRNA group had no significant difference in the two indexes (both $P > 0.05$, Fig. 6).

4. Discussion

We detected the expression of PDRG1 in this work, and found significantly higher levels of PDRG1 in gastric cancer patients with lower cell differentiation, higher tumor stage and more advanced lymph node metastasis, which was in consistency with the finding in the work by Jiang et al. [8]. Similarly, the increased PDRG1 was also proved to be significantly associated with the tumor development of bladder cancer [25], suggesting a close relationship between PDRG1 expression and gastric cancer progression. Previously, human *PDRG1* gene, located on chromosome 20q11.2, is mainly distributed in the cytoplasm, which is poorly expressed in many normal tissues, like liver, stomach, and skeletal muscle [13]. Of note, this site has highly frequent gene amplification in many malignant tumors [26], which may partially explain the up-regulation of PDRG1 in gastric cancer. Apart from that, there was evidence suggesting that the deletion of p53 could increase the expression of PDRG1, while exogenous p53 can down-regulate the activity of PDRG1 promoter to inhibit its expression [13]. Moreover, p53 was found absent in the majority of tumors, including gastric cancer [24], indicating that inactivation of p53 may also contribute to the up-regulation of PDRG1.

In addition, the gastric cancer cell line BGC-823 cell was chosen in this study to conduct transfection experiments *in vitro*. Then we identified that PDRG1 gene silencing led to the reduction of cell proliferation, migration and invasion, and the promotion of cell apoptosis with the arrested cell cycle in the G2/M phase, indicating the beneficial effects of PDRG1 inhibition on gastric cancer patients. At the same time, accumulating evidence from other cancer investigations also indirectly suggested that knockout of PDRG1 could effectively influence the proliferative ability of human colon cancer cells [8]. As performed by Wang J et al., miR-214 can hinder the invasion and migration of bladder cancer cells through its target inhibition of PDRG1 [25]. To further demonstrate this hypothesis, the BGC-823 cells were also treated with ATM inhibitor KU55933, which turned out to be significantly reversed the effect of PDRG1 siRNA on gastric cancer cells. Coincidentally, Tao Z et al. found that inhibition of PDRG1 in lung cancer cells also resulted in the activation of ATM-P53 pathway, and thereby suppressing cancer cell growth and promoting cancer cell apoptosis [23], which provided the possibility that PDRG1 may also

play its role in gastric cancer via modulation of ATM/p53 pathway. In this regard, we detected the expression of ATM/p53 signaling pathway-related proteins. It's not at all surprising that PDRG1 siRNA could not only exert significant inhibition to the protein expression of PDRG1, but also improve the expression levels of p-ATM, p53, p21, p-cdc2 and cleaved caspase-3. It is well-known that most tumors are always occurred owing to the inaccurate repair of DNA damage, which can induce the activation, or abnormal over-expression of oncogenes to cause abnormal cell proliferation [9]. To date, p53 has been proposed as a candidate antioncogene, and after identifying DNA damage signals, cells can activate p53 through various pathways to produce a variety of physiological effects [30]. By activating its downstream target genes, p53 can regulate many biological processes, including cell cycle arrest, cell apoptosis or repair of DNA damage, and the apoptosis procedure would be initiated to induce cell apoptosis if the damage was irrecoverable [2–4]. Moreover, the phosphorylated ATM has been shown to participate in DNA repair by activating different substrate proteins, including anti-oncogene p53 [20]. More than a decade of research has established the ATM kinase as a key molecule that activates p53 after DNA damage [5]. ATM is related to the phosphoSerine-15 p53, which is significant to stabilization, up-regulation, and functional activation of p53 protein [1]. In lung cancer cells, previous study has confirmed that silencing PDRG1 could induce cell apoptosis and cycle arrest via up-regulating ATM and p53 expression [23]. Thus, we speculated that PDRG1 knockdown may increase p53 expression by up-regulating the ATM expression.

Specifically, ATM phosphorylation activates p53, which as a transcription factor would activate the expression of proteins like p21 and GADD45 to induce the phosphorylation of cyclin cdc2 [16]. To our knowledge, the dephosphorylation of cdc2 and cyclin b1 is of great implication for cells to go through the G2/M phase, and accumulated p-cdc2 often leads to the reduced formation of dephosphorylated cdc2 and cyclin b1 compound, which ultimately contributes to arresting cells in G2/M phase of cell cycle [15]. Additionally, it was previously reported that p53-mediated apoptosis involved the co-ordination between the p53 transcription-dependent/-independent pathways [10]. For example, p53 can induce cell apoptosis in cytoplasm through a transcription-independent pathway [6], and it can interact directly with Bak, leading to the release of Cytochrome C [33]. When Cytochrome C was released into the cytoplasm, it can combine with Caspase-9 to form apoptotic bodies, eventually giving rise to the activation of Caspase-3 and the occurrence of cell apoptosis [27]. This observation was also supported by Zhang et al. that PDCD2-mediated ATM/p53 pathway plays an indispensable role in cell cycle arrest and the apoptosis of gastric cancer cells [31]. Based on the described above and other similar reports, we suggested that silencing PDRG1 may regulate downstream cell cycle and apoptotic protein expression by activating the ATM/p53 signaling pathway, thus promoting cell apoptosis, causing cell cycle arrest, and reducing the proliferative, migrating and invasive abilities of BGC-823 cells. Last but not least, we successfully established the tumor xenograft model in nude mice to further come to a conclusion that silencing PDRG1 can significantly constrain tumor volume and reduce tumor weight *in vivo*. Similarly, Tao Z et al. also found that inhibition of PDRG1 could block the growth of lung cancer cells in nude mice and increase the radio-resistance in lung cancer [23]. Taken together, findings of these studies may be used as evidence of the hypothesis that silencing PDRG1 *in vivo* can negatively regulate the growth and proliferation of gastric cancer cells.

This investigation showed that PDRG1 silencing could effectively inhibit cell proliferation, reduce cell migration and invasiveness, and promote cell apoptosis of gastric cancer cells by activating the ATM/p53 signaling pathway.

Declaration of competing interests

None.

Acknowledgements

We would like to express our appreciation to all the reviewers for their suggestive instructions.

References

[1] L.J. Bean, G.R. Stark, Phosphorylation of serines 15 and 37 is necessary for efficient accumulation of p53 following irradiation with UV, Oncogene 20 (2001) 1076–1084.
[2] M.T. Boyd, N. Vlatkovic, p53: a molecular marker for the detection of cancer, Expert Opin. Med. Diagn. 2 (2008) 1013–1024.
[3] C.J. Brown, S. Lain, C.S. Verma, A.R. Fersht, D.P. Lane, Awakening guardian angels: drugging the p53 pathway, Nat. Rev. Cancer 9 (2009) 862–873.
[4] C. Chao, S. Saito, J. Kang, C.W. Anderson, E. Appella, Y. Xu, p53 transcriptional activity is essential for p53-dependent apoptosis following DNA damage, EMBO J. 19 (2000) 4967–4975.
[5] Q. Cheng, J. Chen, Mechanism of p53 stabilization by ATM after DNA damage, Cell Cycle 9 (2010) 472–478.
[6] D.R. Green, G. Kroemer, Cytoplasmic functions of the tumour suppressor p53, Nature 458 (2009) 1127–1130.
[7] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, CA. Cancer J. Clin. 61 (2011) 69–90.
[8] L. Jiang, X. Luo, J. Shi, H. Sun, Q. Sun, M.S. Sheikh, Y. Huang, PDRG1, a novel tumor marker for multiple malignancies that is selectively regulated by genotoxic stress, Cancer Biol. Ther. 11 (2011) 567–573.
[9] P. Kalisperati, E. Spanou, I.S. Pateras, P. Korkolopoulou, A. Varvarigou, I. Karavokyros, V.G. Gorgoulis, P.G. Vlachoyiannopoulos, S. Sougioultzis, Inflammation, DNA damage, helicobacter pylori and gastric tumorigenesis, Front. Genet. 8 (2017) 20.
[10] K. Kojima, M. Konopleva, T. McQueen, S. O'Brien, W. Plunkett, M. Andreeff, Mdm2 inhibitor Nutlin-3a induces p53-mediated apoptosis by transcription-dependent and transcription-independent mechanisms and may overcome Atm-mediated resistance to fludarabine in chronic lymphocytic leukemia, Blood 108 (2006) 993–1000.
[11] Y. Li, D.Q. Yang, The ATM inhibitor KU-55933 suppresses cell proliferation and induces apoptosis by blocking Akt in cancer cells with overactivated Akt, Mol Cancer Ther 9 (2010) 113–125.
[12] J. Liu, Y.S. Xie, F.L. Wang, L.J. Zhang, Y. Zhang, H.S. Luo, Cytotoxicity of 5-Aza-2'-deoxycytidine against gastric cancer involves DNA damage in an ATM-P53 dependent signaling pathway and demethylation of P16(INK4A), Biomed. Pharmacother. 67 (2013) 78–87.
[13] X. Luo, Y. Huang, M.S. Sheikh, Cloning and characterization of a novel gene PDRG that is differentially regulated by p53 and ultraviolet radiation, Oncogene 22 (2003) 7247–7257.
[14] A. Marechal, L. Zou, DNA damage sensing by the ATM and ATR kinases, Cold Spring Harb. Perspect. Biol. 5 (2013).
[15] S. Meng, Y. Zhu, J.F. Li, X. Wang, Z. Liang, S.Q. Li, X. Xu, H. Chen, B. Liu, X.Y. Zheng, L.P. Xie, Apigenin inhibits renal cell carcinoma cell proliferation, Oncotarget 8 (2017) 19834–19842.
[16] A. Nakamizo, T. Inamura, S. Inoha, T. Amano, H. Ochi, K. Ikezaki, M. Fukui, Suppression of Cdc2 dephosphorylation at the tyrosine 15 residue during nitrosourea-induced G2M phase arrest in glioblastoma cell lines, J. Neurooncol. 59 (2002) 7–13.
[17] M.A. Pajares, PDRG1 at the interface between intermediary metabolism and oncogenesis, World J. Biol. Chem. 8 (2017) 175–186.
[18] M.C. Poirier, Chemical-induced DNA damage and human cancer risk, Discov. Med. 14 (2012) 283–288.
[19] J. Shi, P.K. Wei, S. Zhang, Z.F. Qin, J. Li, D.Z. Sun, Y. Xiao, Z.H. Yu, H.M. Lin, G.J. Zheng, X.M. Su, Y.L. Chen, Y.F. Liu, L. Xu, OB glue paste technique for establishing nude mouse human gastric cancer orthotopic transplantation models, World J. Gastroenterol. 14 (2008) 4800–4804.
[20] Y. Shiloh, Y. Ziv, The ATM protein kinase: regulating the cellular response to genotoxic stress, and more, Nat. Rev. Mol. Cell Biol. 14 (2013) 197–210.
[21] V.V. Subhash, S.H. Tan, M.S. Yeo, F.L. Yan, P.C. Peethala, N. Liem, V. Krishnan, W.P. Yong, ATM expression predicts veliparib and irinotecan sensitivity in gastric cancer by mediating P53-independent regulation of cell cycle and apoptosis, Mol. Cancer Ther. 15 (2016) 3087–3096.
[22] T. Sugai, W. Habano, N. Uesugi, Y.F. Jiao, S.I. Nakamura, T. Yoshida, T. Higuchi, Frequent allelic imbalance at the ATM locus in DNA multiploid colorectal carcinomas, Oncogene 20 (2001) 6095–6101.
[23] Z. Tao, S. Chen, G. Mao, H. Xia, H. Huang, H. Ma, The PDRG1 is an oncogene in lung cancer cells, promoting radioresistance via the ATM-P53 signaling pathway, Biomed. Pharmacother. 83 (2016) 1471–1477.
[24] S. Uchino, M. Noguchi, A. Ochiai, T. Saito, M. Kobayashi, S. Hirohashi, p53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer, Int. J. Cancer 54 (1993) 759–764.
[25] J. Wang, X. Zhang, L. Wang, Y. Yang, Z. Dong, H. Wang, L. Du, C. Wang, MicroRNA-214 suppresses oncogenesis and exerts impact on prognosis by targeting PDRG1 in bladder cancer, PLoS One 10 (2015) e0118086.
[26] Q. Wang, B. Wen, G. Yan, J. Wei, L. Xie, S. Xu, D. Jiang, T. Wang, L. Lin, J. Zi, J. Zhang, R. Zhou, H. Zhao, Z. Ren, N. Qu, X. Lou, H. Sun, C. Du, C. Chen, S. Zhang, F. Tan, Y. Xian, Z. Gao, M. He, L. Chen, X. Zhao, P. Xu, Y. Zhu, X. Yin, H. Shen, Y. Zhang, J. Jiang, C. Zhang, L. Li, C. Chang, J. Ma, G. Yan, J. Yao, H. Lu, W. Ying,

F. Zhong, Q.Y. He, S. Liu, Qualitative and quantitative expression status of the human chromosome 20 genes in cancer tissues and the representative cell lines, J. Proteome Res. 12 (2013) 151–161.
[27] S. Wolff, S. Erster, G. Palacios, U.M. Moll, p53's mitochondrial translocation and MOMP action is independent of puma and bax and severely disrupts mitochondrial membrane integrity, Cell Res. 18 (2008) 733–744.
[28] Y. Xu, Y. Diao, S. Qi, X. Pan, Q. Wang, Y. Xin, X. Cao, J. Ruan, Z. Zhao, L. Luo, C. Liu, Z. Yin, Phosphorylated Hsp27 activates ATM-dependent p53 signaling and mediates the resistance of MCF-7 cells to doxorubicin-induced apoptosis, Cell. Signal. 25 (2013) 1176–1185.
[29] C.L. Yuan, H. Li, L. Zhu, Z. Liu, J. Zhou, Y. Shu, Aberrant expression of long non-coding RNA PVT1 and its diagnostic and prognostic significance in patients with gastric cancer, Neoplasma 63 (2016) 442–449.
[30] H.J. Yun, S.K. Hyun, J.H. Park, B.W. Kim, H.J. Kwon, Widdrol activates DNA damage checkpoint through the signaling Chk2-p53-Cdc25A-p21-MCM4 pathway in HT29 cells, Mol. Cell. Biochem. 363 (2012) 281–289.
[31] J. Zhang, W. Wei, H.C. Jin, R.C. Ying, A.K. Zhu, F.J. Zhang, Programmed cell death 2 protein induces gastric cancer cell growth arrest at the early S phase of the cell cycle and apoptosis in a p53-dependent manner, Oncol. Rep. 33 (2015) 103–110.
[32] Q. Zhao, J. Fan, W. Hong, L. Li, M. Wu, Inhibition of cancer cell proliferation by 5-fluoro-2'-deoxycytidine, a DNA methylation inhibitor, through activation of DNA damage response pathway, Springerplus 1 (2012) 65.
[33] J.T. Zilfou, S.W. Lowe, Tumor suppressive functions of p53, Cold Spring Harb. Perspect. Biol. 1 (2009) a001883.
[34] Q.F. Zuo, L.Y. Cao, T. Yu, L. Gong, L.N. Wang, Y.L. Zhao, B. Xiao, Q.M. Zou, MicroRNA-22 inhibits tumor growth and metastasis in gastric cancer by directly targeting MMP14 and snail, Cell Death Dis. 6 (2015) e2000.