Tanshinone I and simvastatin inhibit melanoma tumour cell growth by regulating poly (ADP ribose) polymerase 1 expression

YUYAN ZHANG[1], JIUSUI HUANG[1], YAPENG HUANG[2], SHIKE ZHANG[2], WEIZHOU WU[2], HUI LONG[1], XIAOLU DUAN[2], YONGCHANG LAI[3] and WENQI WU[2]

[1]Department of Pharmacy, Guangzhou Institute of Dermatology, Guangzhou, Guangdong 510095; [2]Department of Urology, Minimally Invasive Surgery Centre, Guangzhou Urology Research Institute, Guangdong Key Laboratory of Urology, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510230; [3]Department of Urology, The Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen, Guangdong 518033, P.R. China

Received December 3, 2019; Accepted August 3, 2020

DOI: 10.3892/mmr.2020.11678

Abstract. Melanoma is one of the most aggressive forms of skin tumour with poor prognosis; no effective therapy has been established for melanoma at the metastatic stage. The present study aimed to investigate the role of poly (ADP ribose) polymerase (PARP) inhibitors (PARPis) and PARP1 expression in melanoma progression. In addition, whether high PARP1 expression was associated with poor overall survival in melanoma, and whether a combination effect existed between PARPis and other anti-tumour compounds (e.g., sunitinib) was analysed. The PARP1 expression was detected using western blot analysis and the proliferation of cells was detected with a colony formation assay. In addition, cell viability assays and xenograft tumor experiments were conducted. The results of the present study demonstrated that sunitinib reduced PARP1 expression and proliferation of melanoma cells. Notably, one of the PARPis, veliparib, reversed the inhibitory effect of sunitinib on PARP1 expression and proliferation, indicating that inhibition of PARP1 enzyme activity by PARPi may be different from the inhibition of PARP1 expression in melanoma cell biological function. To further confirm the relationship between PARP1 expression and tumour cell proliferation, seven compounds, including common approved drugs and natural Chinese medicine monomers, were screened, and the results demonstrated that simvastatin and tanshinone I exerted an inhibitory effect on PARP1 expression and melanoma cell proliferation, and their combination was more effective than simvastatin alone *in vivo*. The results indicated that simvastatin and tanshinone I inhibited melanoma and renal tumour cells by regulating PARP1 expression, and in addition to the enzyme activity of PARP1, the expression of PARP1 protein may serve a role in tumour progression.

Correspondence to: Dr Yongchang Lai, Department of Urology, The Eighth Affiliated Hospital, Sun Yat-sen University, 3025 Shennan Middle Road, Futian, Shenzhen, Guangdong 518033, P.R. China
E-mail: gkim89882000@163.com

Dr Wenqi Wu, Department of Urology, Minimally Invasive Surgery Centre, Guangzhou Urology Research Institute, Guangdong Key Laboratory of Urology, The First Affiliated Hospital of Guangzhou Medical University, 1 Kangda Road, Haizhu, Guangzhou, Guangdong 510230, P.R China
E-mail: wwqwml@163.com

Key words: tanshinone I, poly (ADP ribose) polymerase 1, simvastatin, melanoma, veliparib, sunitinib

Introduction

Poly (ADP ribose) polymerase 1 (PARP1) is involved in the base excision pathway of DNA repair in most eukaryotic cells. As a part of this pathway, PARP serves a key role in the maintenance of DNA integrity (1). The PARP family consists of 17 subtypes, of which PARP1 is the most abundant and ubiquitous member that participates in various functions performed by this family (2). Increased expression of PARP1 has been reported to be an independent negative prognostic marker in mucosal melanomas (3). PARP1 recruits Kruppel-like factor 4 to activate telomerase expression in cancer and stem cells (4). The common PARP inhibitors (PARPis) include olaparib, veliparib and rucaparib. Besides breast cancer gene (BRCA) mutation-associated cancer, the benefits of PARPis in earlier treatment settings, including neoadjuvant, adjuvant and promising combination therapeutic strategies, such as those with other DNA damage response inhibitors and immune checkpoint inhibitors, are of increasing interest (5). However, to date, PARPi treatment is based on the lethal synthesis theory and is restricted to patients with BRCA1/2 mutation-associated breast and ovarian cancer (6).

Melanoma is a heterogeneous disease and one of the most aggressive forms of skin tumour with poor prognosis (7). Melanoma is associated with numerous genetic mutations or alterations in signalling pathways (e.g. BRAF), and there is no effective therapy for melanoma at the metastatic stage (e.g. uveal melanoma) (8). Veliparib has been reported to be effective in the treatment of BRAF inhibitor-resistant

melanoma cells (9). However, while a randomized phase II study of veliparib with temozolomide demonstrated a higher median progression-free survival for veliparib compared with that of the placebo, the difference was not statistically significant (10). Besides, while olaparib increased response to dacarbazine (an alkylating agent) in uveal melanoma (8), both veliparib and olaparib combined increased the sensitivity of various histological subtypes of single-nucleotide polymorphism (SNP)-carrier cancer cells to alkylating agents, without an effect on wild-type cells (11).

Except for immunotherapy, single drugs directed to single targets have not made much progress in the treatment of tumours. Danshen, a common traditional Chinese medicine used in clinical practice, contains multiple components (e.g. tanshinone I, tanshinone IIA and tanshinone IIB) and has multiple targets (12). The effects of tanshinone IIA are well studied, particularly in tumours (13,14). In addition, the role of tanshinone I in tumour proliferation, metastasis and drug resistance has also been studied (15,16). However, to some extent, its weak potency and poor drug-like properties restrict its clinical development as a cancer therapy (17).

PARPis are designed to modulate enzyme activity without affecting the expression of PARP1. Increased PARP1 gene expression in tumours has been shown to be associated with melanoma ulceration and poorer overall survival (OS) (18). Our previous study in prostate cancer demonstrated that inhibition of PARP1 expression significantly reduced prostate cancer cell proliferation and migration irrespective of BRCA1/2 mutations (19). However, in vascular smooth muscle cells and endothelial cells, PARP1 inhibition may be protective against apoptosis and/or necrosis in response to H_2O_2 or tumour necrosis factor (20). Thus, the exact role of PARPis and PARP1 expression in wild-type or SNP-containing melanoma cells requires further elucidation. The present study explored the role of PARPis and PARP1 in tumour progression, and screened for compounds that significantly promoted melanoma efficacy and modulated PARP1 expression to provide a potential basis for assessing related drugs for targeting PARP1 in melanoma.

Materials and methods

Materials. Sunitinib, veliparib, olaparib, mefloquine, simvastatin, dihydroartemisinin, tanshinone I, cryptotanshinone, gossypol and docetaxel were purchased from Selleck Chemicals. Anti-FoxO3a (cat. no. 12829), anti-α/β-tubulin (cat. no. 2148) and anti-Bcl-2 (cat. no. 15071) antibodies were purchased from Cell Signaling Technology, Inc. phosphorylated (p)FoxO3a were from Abcam Inc. (cat. no. ab154786). Anti-PARP1 (cat. no. sc-8007) was obtained from Santa Cruz Biotechnology, Inc. Tanshinone I, dioscin and simvastatin were obtained from Shanghai YuanYe Biotechnology Co., Ltd. DMEM, RPMI-1640, MEM and FBS were obtained from Gibco; Thermo Fisher Scientific, Inc. MTS was purchased from Shanghai BestBio (cat. no. BB-4204-3). The secondary fluorescent antibodies, IRDye 800CW goat anti-mouse (cat. no. 926-32210) and IRDye 800CW goat anti-rabbit (cat. no. 926-32211) were from were from LI-COR. All other laboratory reagents in common use were of domestic analytical pure grade.

OS analysis. OS data for PARP1 and PARP2 expression, and prognosis for skin cutaneous melanoma (SKCM) and uveal melanoma (UVM) were obtained from the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn) by setting the percentage of the Cut off-Low and Cut off-High according to the gene and tumor subtype (21).

Cell lines and cultures. Melanoma cell lines (A-375) and renal tumour cell lines (769-P, Caki-1 and ACHN) were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. The cell lines were cultured in 90% DMEM (A-375 cells), RPMI-1640 (769-P and Caki-1 cells) or MEM (ACHN cells) supplemented with 10% FBS at 37˚C with 5% CO_2 and saturated humidity.

Cell viability tests. The effects of tanshinone I and simvastatin, and veliparib or olaparib alone or in combination with sunitinib or docetaxel (24, 48 and 72 h) on A-375 cell proliferation were assessed using the MTS assay. Briefly, after the cells were seeded in 96-well plates (3,000-5,000 per well), the corresponding drugs were added the next day and incubated in 37˚C for 24-72 h before the medium was replaced with an MTS mixture (10 μl/well) in 37˚C for 1-3 h. The optical values were measured using a multimode reader (BioTek Instruments, Inc.) at 490 nm. The control group was normalized to '100', and the relative survival rates were then calculated.

Colony formation experiment. Cell (A-375, ACHN, 769-P or Caki-1) suspensions were seeded in 6-well dishes at ~$1x10^3$ cells/well and incubated in the CO_2 incubator at 37˚C for 24 h. The medium was replaced with fresh medium containing the simvastatin or tanshinone I (with a concentration of 0, 1, 3 and 10 μM). When macroscopic clones were observed in the dish after 10-15 days, the cells were washed twice with PBS, and then fixed and dyed with 4% crystal violet in alcohol for 20-30 min in room temperature. After the dye solution was washed with distilled water and air-dried, images of the cells were captured using a camera directly, and the number of cells in the different groups were counted manually or using Image-Pro Plus 16.0 (Media Cybernetics, Inc.).

Morphological analysis. After the cells were treated by drugs for 24 h, images of the cells were captured under a fluorescent microscope in ordinary light (magnification, x20), when the morphological features of cells changed significantly.

Western blotting. After the A-375 cells were treated with the same concentration (10 μM) of drugs (valiparib, sunitinib, mefloquine, simvastatin, dihydroartemisinin, tanshinone I, cryptotanshinone, gossypol and dioscin) for 24 h in the CO_2 incubator at 37˚C, cells were washed three times with PBS, and radioimmunoprecipitation assay lysis buffer (Nanjing KeyGen Biotech Co., Ltd.; cat. no. KGP702-100) was added to extract total protein. The cells were then scraped and the lysate was collected. After the cells in the eppendorf tube were inserted into the ice, they were broken using a ultrasonic cell ddisruptorr (150W/20KHz; Sonics and Materials, Inc.) 3-5 times (5 sec each time, with a 45% amplitude), the lysates were centrifuged at 12,000 x g for 30 min at 4˚C and the supernatants were retained. The protein concentrations

were quantified using a bicinchoninic acid kit (Thermo Fisher Scientific, Inc.). Protein samples were separated by SDS-PAGE with a 10% gel and transferred to polyvinylidene difluoride membranes. The membranes were cut and blocked with 5% skimmed milk for 1-2 h in the room temperature and incubated overnight at 4˚C with the primary antibodies of the PARP-1 (1:200), FoxO3a (1:1,000), p-FoxO3a (1:1,000), tubulin (1:1,000) and anti-Bcl-2 (1:1,000). After washing with PBS, the membranes were incubated in fluorescently labelled secondary antibodies (1:7,500) for 1-2 h at room temperature. The membranes were then washed with PBS and directly scanned on an LI-COR Image Studio Ver5.2 imaging system (without other visualization reagents). The grey values of the western blot bands were analysed using ImageJ (National Institutes of Health; version 1.4.3.67).

Xenograft tumour model. The animal experiments were approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (Guangzhou, China). A total of 16 male BALB/c nude mice (weight 18-22 g; age, ~4 weeks old) were obtained from the Guangdong Medical Laboratory Animal Centre. The mice were allowed to acclimatise for a week in a specific pathogen free environment [temperature, 20-26˚C; relative humidity, 40-70%; with a high pressure disinfection equipment, purified water system, and automatic switching on (at 7 a.m.) and off (at 7 p.m.) light system]. Each cage had a drinking bottle for the water and the food was supplied on the other side of the lid. The mice had common food and drank water freely. A subcutaneous xenograft tumour model of A-375 cells was established in nude mice. A-375 cells (~$2x10^6$) in a 150-μl suspension with 20% Matrigel (Corning, Inc.; cat. no. 354248) were subcutaneously injected after the mice were anesthetized by intraperitoneal injection of 40-60 mg/kg sodium pentobarbital. After 8 days, 15 tumour-bearing mice were selected for subsequent experiments; one mouse was excluded due to relatively slow tumorigenesis. The 15 mice were equally divided into three groups: i) Control (solvent, DMSO); ii) 10 mg/kg tanshinone I; and iii) 10 mg/kg tanshinone I and 20 mg/kg simvastatin, and were administered intraperitoneal injection from days 8 to 26, with the tumour size measured every 4-5 days. On day 30, mice were sacrificed with excessive intraperitoneal injection of sodium pentobarbital (three times that of the anaesthetic dose), and the tumour volume and weight of the nude mice were observed and calculated. The tumour volume was estimated using the following formula: Volume = $(a \times b^2)/2$; ‘a’ represent the longest diameters, ‘b’ represent the shortest diameters. The humane endpoint set for this study was that, when the maximum tumour size of the mice was up to ~1,200 mm^3, all the nude mice were sacrificed 4 days later and then the tumor volume were measured.

Molecular characteristics and medicinal properties. The two-dimensional and three-dimensional structures and other molecular characteristics of simvastatin and tanshinone I were obtained using PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Statistical analysis. All of the experiments had a minimum of three replicates. Data are presented as the mean ± SEM and were analysed by one-way analysis of variance followed by a Tukey's (homogeneity of variance) or Dunnett's T3 (heterogeneity of variance) post hoc test. All data were analysed using SPSS 16.0 (SPSS, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Background and precise processes of this research. Our previous study demonstrated that PARP1 small interfering RNA inhibited prostate cancer cell proliferation (19). The present study assessed the association between PARP1 expression and melanoma prognosis. Data from the GEPIA database demonstrated that high PARP expression was correlated with poor OS in melanoma (Fig. 1). However, no significant differences were observed between the effects of PARPis (1-10 μM) on melanoma cells (Fig. 2). Testing of the combination of angiogenesis inhibitors with PARPis demonstrated that veliparib reversed the inhibitory effect of sunitnib, an angiogenesis inhibitor, on A-375 cells (Fig. 2). Thus, instead of PARPis, this study focused on the role of PARP1 expression in tumours. The present study screened for compounds that significantly inhibited PARP1 expression and explored the role of PARP1 in melanoma progression to provide a basis for assessing PARP1 as a tumour target.

High PARP1 expression is associated with melanoma OS. The relationship between PARP expression and melanoma prognosis was analysed. Data from the GEPIA database demonstrated that high PARP expression was associated with poor OS in melanoma (Fig. 1). Notably, high PARP1 expression was associated with poor OS in SKCM (Fig. 1A) and UVM (Fig. 1B). Additionally, high PARP2 expression was associated with a poor OS in UVM (Fig. 1C).

PARPis reverse the inhibitory effect of sunitinib on PARP1 expression and melanoma cell proliferation. After evaluating the association between PARP1 and melanoma prognosis, the effects of PARPis on melanoma cells were explored. The results of the present study demonstrated that veliparib (1-10 μM) and olaparib (1-10 μM) had no significant effect on melanoma cell survival (Fig. 2A and B). Although 30 μM veliparib had a partial inhibitory effect on melanoma cell survival, but this concentration appears high for an antitumour drug. In addition, the combination of PARPis and other anti-tumour drugs was tested. As an antiangiogenic drug, sunitinib may have activity in patients with melanoma and KIT mutations (22). Thus, the effects of combining sunitinib, an angiogenesis inhibitor, and PARPis on melanoma cell growth and PARP1 expression were evaluated. The results demonstrated that sunitinib significantly inhibited melanoma cell proliferation and that veliparib reversed this inhibitory effect on A-375 cells (Fig. 2C). Similarly, another PARPi, olaparib, also significantly reversed the inhibitory effect of sunitinib on melanoma cell proliferation (Fig. 2C). In addition, sunitinib significantly inhibited PARP1 expression in melanoma cells, an effect that was significantly reversed by veliparib without significantly affecting the expression of Bcl-2, FoxO3a or pFoxO3a (Fig. 2D). Additionally, veliparib partly reversed the inhibitory effect of docetaxel, a tubulin polymerization

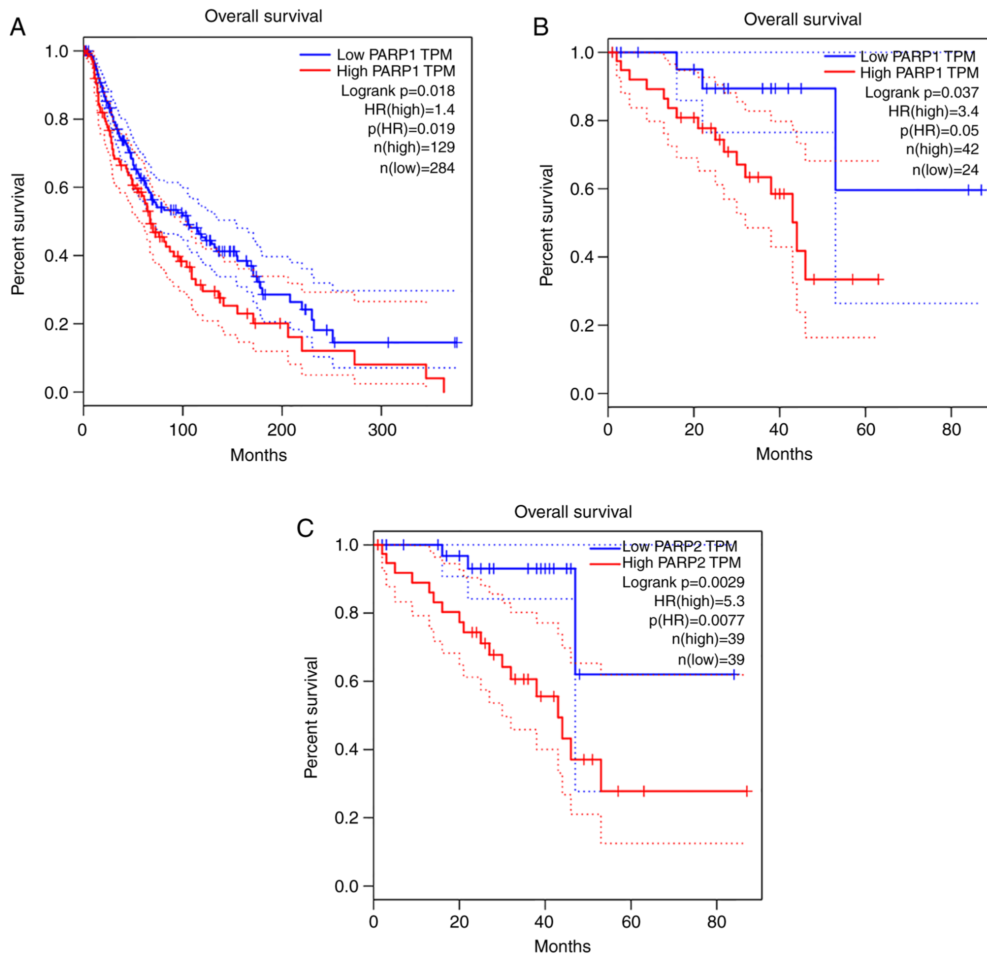

Figure 1. High PARP1 expression is associated with OS in melanoma. High PARP1 expression was associated with poor OS in (A) skin cutaneous melanoma and (B) UVM. (C) High PARP2 expression was associated with OS in UVM. OS, overall survival; UVM, uveal melanoma. P-values represent the results of the log-rank test.

promoter, on melanoma cell proliferation (Fig. 2E). These results suggested that PARP1 expression may be associated with melanoma cell proliferation or survival.

Tanshinone I and simvastatin exert inhibitory effects on melanoma A-375 cell proliferation and PARP1 expression. To further confirm the relationship between PARP1 expression and melanoma cells, the present study screened for drugs that significantly inhibited PARP1 expression among common drugs and traditional Chinese medicine monomers (mefloquine, simvastatin, dihydroartemisinin, tanshinone I, cryptotanshinone, gossypol and dioscin). The results demonstrated that tanshinone I and simvastatin exerted a notable inhibitory effect on PARP1 expression in melanoma A-375 cells (Fig. 3A).

The effects of tanshinone I and simvastatin on the molecular biological function of melanoma cells *in vitro* were investigated. Compared with that of the control group, tanshinone I significantly inhibited A-375 cell proliferation (Fig. 3B) and colony formation (Fig. 3C). By comparing the druggability of the tanshinone I and simvastatin, simvastatin, which is already a clinically used drug, was used to assess its effects on PARP1 expression. Compared with that of the control group, simvastatin significantly inhibited proliferation (Fig. 3D), PARP1 expression (Fig. 3E) and colony formation (Fig. 3F) in A-375 cells.

Tanshinone I and simvastatin inhibit tumour growth in nude mice. Simvastatin notably affected the morphology of A-375 cells, which were markedly rounded with characteristic morphological changes of apoptosis (Fig. 4A). In addition to melanoma cells, the results also demonstrated that tanshinone I significantly inhibited colony formation in 769-P renal cancer cells (Fig. 4B), Caki-1 cells (Fig. 4C) and ACHN cells (Fig. 4D). Subsequently, the combined effect of tanshinone I and simvastatin on melanoma was explored *in vivo*. The combination of tanshinone I and simvastatin inhibited the growth of tumour xenografts formed by A-375 cells in nude mice compared with that of the control group (Fig. 4E). Finally, in order to examine the molecular characteristics and differences of the two compounds, the two-dimensional and three-dimensional structures of simvastatin and tanshinone I were obtained using PubChem (https://pubchem.ncbi.nlm.nih.gov/), as demonstrated in Fig. 4F and G. Based on the molecular characteristics and medicinal properties of tanshinone I and simvastatin, the combination of simvastatin and tanshinone I may exert an inhibitory effect on tumour progression.

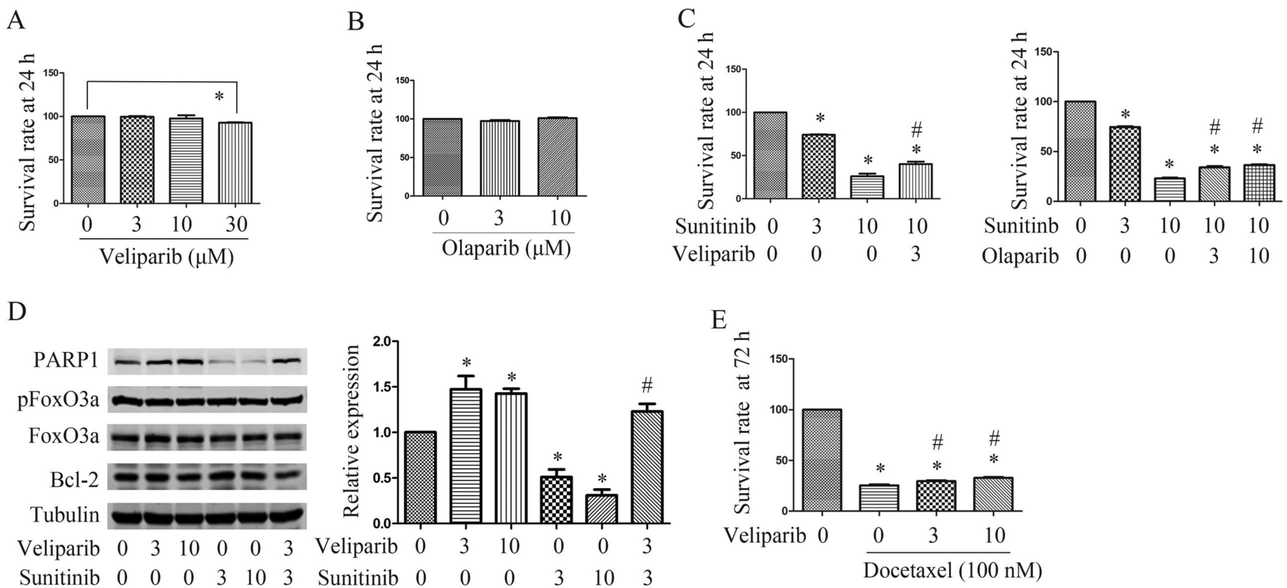

Figure 2. Veliparib significantly reverses the inhibitory effect of sunitinib on PARP1 expression and melanoma cell viability. Effect of (A) veliparib (3, 10 and 30 μM) and (B) olaparib (3 and 10 μM) on melanoma cell viability after 24 h. (C) Effects of PARP inhibitors (veliparib and olaparib) on sunitinib-induced inhibition of melanoma cell viability at 24 h. (D) Effect of veliparib on sunitinib-induced inhibition of PARP1 expression in melanoma cells. (E) Effect of veliparib on docetaxel-induced inhibition of melanoma cell viability and proliferation at 72h. *P<0.05 vs. control group; #P<0.05 vs. corresponding group, C (sunitinib 10 μM), D (sunitinib 3 μM) and E (docetaxel 100 nM). PARP, poly (ADP ribose) polymerase; pFoxO3a, phosphorylated-FoxO3a.

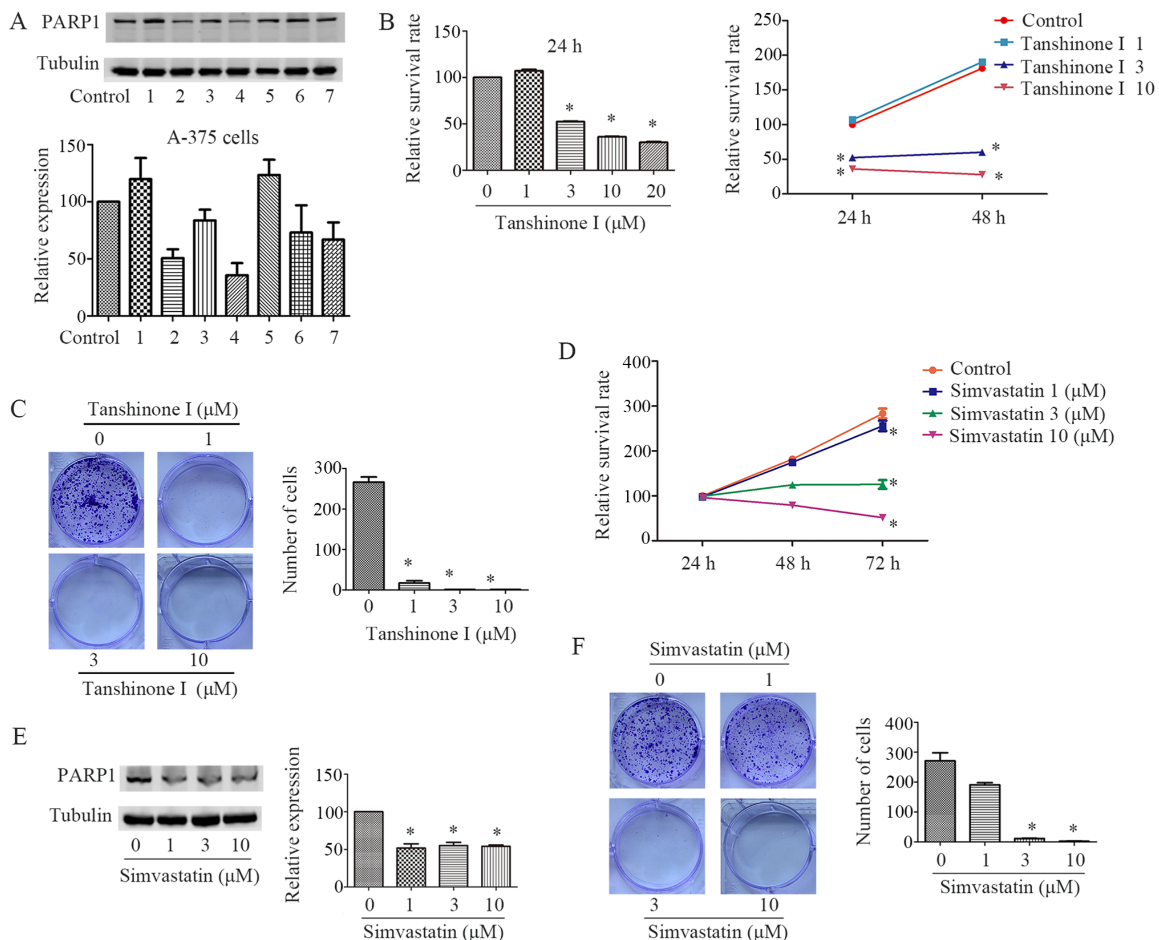

Figure 3. Tanshinone I and simvastatin significantly inhibit proliferation and PARP1 expression in melanoma A-375 cells. (A) Western blotting was used to determine the effects of mefloquine (1), simvastatin (2), dihydroartemisinin (3), tanshinone I (4), cryptotanshinone (5), gossypol (6) and dioscin (7) on PARP1 expression in melanoma A-375 cells. Although no statistical difference were found, a inhibition tendency on PARP1 expression for simvastatin and thanshinone I could be seen in the band. Effects of tanshinone I on A-375 (B) cell proliferation and (C) colony formation. Effects of simvastatin on A-375 (D) cell proliferation, (E) PARP1 expression and (F) colony formation. *P<0.05 vs. corresponding same processing time control group. PARP1, poly (ADP ribose) polymerase 1.

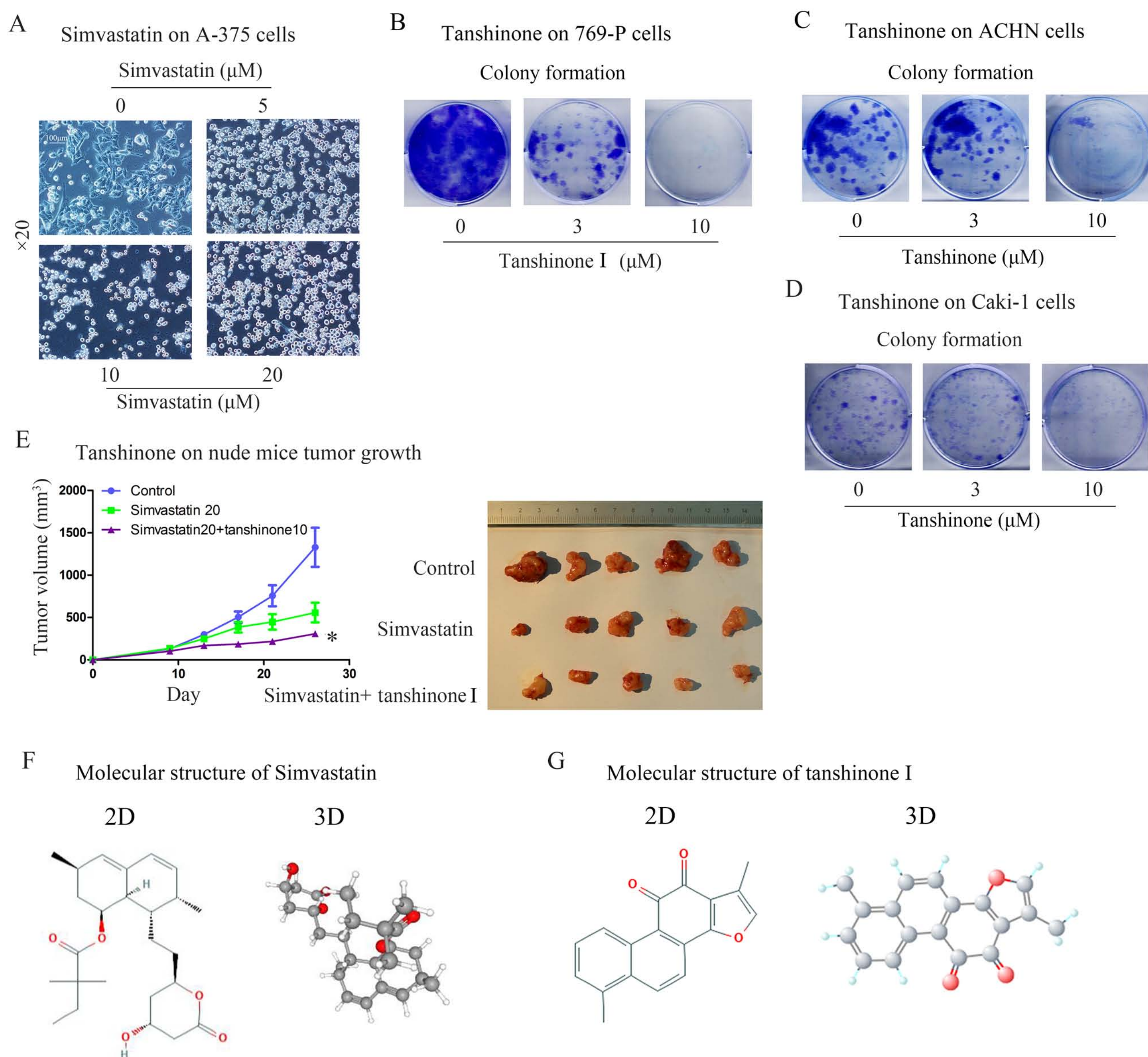

Figure 4. Tanshinone I combined with simvastatin inhibits the growth of transplanted tumours formed by A-375 cells in nude mice. (A) Cell morphology of A-375 cells treated with 0, 5, 10 and 20 μM simvastatin. Magnification, x20. Effect of tanshinone I on colony formation in (B) 769-P, (C) Caki-1 and (D) ACHN cells. (E) Effect of tanshinone I (10 mg/kg) and simvastatin (20 mg/kg) on the growth of tumour xenografts formed by A-375 cells in nude mice. Two- and three-dimensional structures of (F) simvastatin and (G) tanshinone I. *P<0.05 vs. control group. PARP, poly (ADP ribose) polymerase.

Discussion

The current database analysis demonstrated that PARP1 was negatively associated with the OS and prognosis of melanoma. Sunitinib inhibited PARP1 expression and proliferation in A-375 cells, whereas its effects were partly reversed by veliparib. Screening revealed simvastatin and tanshinone I as compounds that inhibited PARP1 expression, and their combination inhibited the growth of xenograft tumours constructed using A-375 cells in nude mice. The results of the present study identified drugs (tanshinone I and simvastatin) that inhibited PARP1 expression, and provided evidence that tanshinone I may improve melanoma tumour cell sensitivity to simvastatin by regulating PARP1 expression.

The identification of specific targets to inhibit tumour cell growth or enhance the efficacy of chemotherapy/radiotherapy provides an important basis for the development of anti-tumour drugs. Although PARPis have been approved for BRCA mutation-associated cancer, their effects on other molecular tumour subtypes and the specific mechanism underlying the effects of PARP on tumour proliferation, invasion and metastasis remain to be further clarified. Tanshinone I is a monomer of *Salvia miltiorrhiza* and inhibits tumour cell proliferation (12); however, its specific mechanism remains to be determined. The present study identified tanshinone I and simvastatin as compounds that exerted inhibitory effects on PARP1 expression, and demonstrated that tanshinone I improved tumour sensitivity to simvastatin.

Various tyrosine kinase inhibitors have been approved for melanoma treatment (23); however, their effectiveness is limited because of drug resistance. A novel benzoxazole compound exhibited synergistic anti-tumour

effects in combination with vemurafenib (a BRAF inhibitor) and docetaxel (24). Unlike O6-alkylguanine DNA alkyltransferase (MGMT)-deficient melanoma cells, an antiproliferative senescent response induced by temozolomide was enhanced by PARPi in MGMT-positive cancer cells (25), which indicates the different functions for PARPi. KIT mutations may serve as an adverse prognostic factor in metastatic melanoma and sunitinib may have activity in patients with melanoma and KIT mutations (22). In the present study, sunitinib significantly inhibited PARP1 expression. However, the combination of a PARPi and sunitinib reduced the inhibitory effects of sunitinib on melanoma cell growth and PARP1 expression. Notably, inhibition of PARP1 activity in the cells by veliparib appeared to stimulate PARP1 expression; this may be associated with the feedback inhibition, similar to the phenomenon of phorbol 12-myristate-13-acetate on tumour necrosis factor-α converting enzyme to some extent (26).

Cells overexpressing Bcl-2 have been reported to exhibit a significantly improved response to salvage radiotherapy compared with that of cells with low Bcl-2 expression (27). In the present study, a PARPi and sunitinib affected PARP1 expression, but did not affect the expression of Bcl-2 and pFoxO3a. The initial PARPis are analogs of nicotinamide designed to compete with nicotinamide adenine dinucleotide at the catalytic pocket of PARP to modulate enzyme activity (28). Detection of PARP1 expression in this study indicated that, besides the enzymatic activity of PARP1, PARP1 expression may be important for carcinogenesis. Olaparib increased the response to dacarbazine, an alkylating agent, in a patient-derived xenograft model of uveal melanoma (8). Although veliparib and olaparib have been reported to increase the sensitivity of various histological subtypes of SNP carrier cancer cells to alkylating agents, they have no effect on wild-type cells (11). The results of the present study demonstrated that veliparib and olaparib had no significant effect on melanoma cell survival. These results were concordant with those reported by a previous study in which clinicians demonstrated resistance to olaparib in patients with cancer (29). However, the sensitivity may also be associated with the tumour type, as breast cancer cells exhibited increased sensitivity to the same concentration of olaparib (30).

The present study screened for compounds that affected PARP1 expression, and preliminarily evaluated the drug potency of these compounds and their combined effect on tumour growth to provide a way of exploring PARP1-targeted anti-tumour drugs. Through experimental verification, tanshinone I and simvastatin provided a good foundation for the development of anti-tumour drugs targeting PARP1 and their chemical structure derivatives are anticipated. However, whether the other targets of simvastatin and tanshinone I were involved in their effects remains to be studied. Therefore, further studies of these compounds are essential for identifying additional related targets for this combined effect. In addition, although proliferation, colony formation and morphology were assessed, the lack of direct apoptosis and cell cycle analyses may be a limitation of the present study.

The doses of statins required for anti-tumour effects are 100- to 500-fold higher than those needed to lower cholesterol levels; therefore, the use of tumour-targeted delivery systems may greatly improve their anti-tumour efficacy (31). In this study, the combination of tanshinone I and simvastatin improved the anti-tumour efficacy of simvastatin, but whether the combined effect of tanshinone I and other types of statins have the same effect requires further assessment. In a previous study, simvastatin increased the anti-tumour activity of paclitaxel (PTX) carried by lipid nanoemulsions (LDE), but not of the commercial PTX (not carried by LDE), possibly because of increased low-density lipoprotein receptor expression by statins that bind and internalize LDE-PTX (32). Thus, other potential mechanisms for the effects of the combination of simvastatin and tanshinone I on melanoma require further exploration. Simvastatin is a prodrug for β-hydroxy β-methylglutaryl-CoA reductase, which is activated by drug-metabolizing enzymes into metabolite *in vivo* (33). Compared with the *in vivo* metabolism, the drug-metabolizing enzyme activity of cancer cells *in vitro* is usually lower. As the results of the present study demonstrated that simvastatin inhibited melanoma cells *in vitro* and *in vivo*, it may be hypothesized that simvastatin and its metabolite exert inhibitory activity on melanoma cells.

In conclusion, the results of the present study demonstrated that tanshinone I and simvastatin significantly inhibited PARP1 expression, and that tanshinone I may effectively improve tumour cell sensitivity to simvastatin. To provide more evidence for the function of tanshinone I on different types of tumour cells, renal cancer cells were selected for functional experiments. Similar to the result on melanoma cells, tanshinone I also inhibited the colony formation of renal cancer cells. These findings suggested that inhibiting PARP1 expression may be a potential method for treatment of melanoma and renal cell carcinoma. The enzyme activity and expression of PARP1 may serve a role in tumour progression by different mechanisms.

Acknowledgements

Not applicable.

Funding

The current study was funded in part by research grants from the Science and Technology Project of Guangzhou Municipal Health and Family Planning Commission (grant no. 20181A011059) and the National Natural Science Foundation of China (grant nos. 81570633 and 81803576).

Availability of data and materials

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

Authors' contributions

YZ and YL performed the majority of experiments, analysed the data and prepared the manuscript. WenqiW designed the project and provided expert advice to the study, and revised the manuscript. JH analysed the survival data of melanoma obtained from the database. YH, SZ and WeizhouW performed

the *in vivo* experiments. HL and XD analysed parts of the data, revised and discussed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The animal experiments were approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kummar S, Chen A, Parchment RE, Kinders RJ, Ji J, Tomaszewski JE and Doroshow JH: Advances in using PARP inhibitors to treat cancer. Bmc Med 10: 25, 2012.
2. Luo X and Kraus WL: On PAR with PARP: Cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev 26: 417-432, 2012.
3. Donizy P, Wu CL, Mull J, Fujimoto M, Chłopik A, Peng Y, Shalin SC, Selim MA, Puig S, Fernandez-Figueras MT, *et al*: Up-regulation of PARP1 expression significantly correlated with poor survival in mucosal melanomas. Cells 9: 1135, 2020.
4. Hsieh MH, Chen YT, Chen YT, Lee YH, Lu J, Chien CL, Chen HF, Ho HN, Yu CJ, Wang ZQ and Teng SC: PARP1 controls KLF4-mediated telomerase expression in stem cells and cancer cells. Nucleic Acids Res 45: 10492-10503, 2017.
5. Pilié PG, Gay CM, Byers LA, O'Connor MJ and Yap TA: PARP inhibitors: Extending benefit beyond BRCA-mutant cancers. Clin Cancer Res 25: 3759-3771, 2019.
6. Bian C, Zhang C, Luo T, Vyas A, Chen SH, Liu C, Kassab MA, Yang Y, Kong M and Yu X: $NADP^+$ is an endogenous PARP inhibitor in DNA damage response and tumor suppression. Nat Commun 10: 693, 2019.
7. Dinnes J, errante di Ruffano L, Takwoingi Y, Cheung ST, Nathan P, Matin RN, Chuchu N, Chan SA, Durack A, Bayliss SE, *et al*: Ultrasound, CT, MRI, or PET-CT for staging and re-staging of adults with cutaneous melanoma. Cochrane Database Syst Rev 7: CD012806, 2019.
8. de Koning L, Decaudin D, El BR, Nicolas A, Carita G, Schuller M, Ouine B, Cartier A, Naguez A, Fleury J, *et al*: PARP inhibition increases the response to chemotherapy in uveal melanoma. Cancers (Basel) 11: 751, 2019.
9. Fratangelo F, Camerlingo R, Carriero MV, Pirozzi G, Palmieri G, Gentilcore G, Ragone C, Minopoli M, Ascierto PA and Motti ML: Effect of ABT-888 on the apoptosis, motility and invasiveness of BRAFi-resistant melanoma cells. Int J Oncol 53: 1149-1159, 2018.
10. Middleton MR, Friedlander P, Hamid O, Daud A, Plummer R, Falotico N, Chyla B, Jiang F, McKeegan E, Mostafa NM, *et al*: Randomized phase II study evaluating veliparib (ABT-888) with temozolomide in patients with metastatic melanoma. Ann Oncol 26: 2173-2179, 2015.
11. Abecassis I, Sedgewick AJ, Romkes M, Buch S, Nukui T, Kapetanaki MG, Vogt A, Kirkwood JM, Benos PV and Tawbi H: PARP1 rs1805407 increases sensitivity to PARP1 inhibitors in cancer cells suggesting an improved therapeutic strategy. Sci Rep 9: 3309, 2019.
12. Ren J, Fu L, Nile SH, Zhang J and Kai G: Salvia miltiorrhiza in treating cardiovascular diseases: A review on its pharmacological and clinical applications. Front Pharmacol 10: 753, 2019.
13. Sui H, Zhao J, Zhou L, Wen H, Deng W, Li C, Ji Q, Liu X, Feng Y, Chai N, *et al*: Tanshinone IIA inhibits β-catenin/VEGF-mediated angiogenesis by targeting TGF-β1 in normoxic and HIF-1α in hypoxic microenvironments in human colorectal cancer. Cancer Lett 403: 86-97, 2017.
14. Munagala R, Aqil F, Jeyabalan J and Gupta RC: Tanshinone IIA inhibits viral oncogene expression leading to apoptosis and inhibition of cervical cancer. Cancer Lett 356: 536-546, 2015.
15. Yan Y, Su W, Zeng S, Qian L, Chen X, Wei J, Chen N, Gong Z and Xu Z: Effect and mechanism of tanshinone I on the radiosensitivity of lung cancer cells. Mol Pharm 15: 4843-4853, 2018.
16. Lee CY, Sher HF, Chen HW, Liu CC, Chen CH, Lin CS, Yang PC, Tsay HS and Chen JJ: Anticancer effects of tanshinone I in human non-small cell lung cancer. Mol Cancer Ther 7: 3527-3538, 2008.
17. Ding C, Tian Q, Li J, Jiao M, Song S, Wang Y, Miao Z and Zhang A: Structural modification of natural product tanshinone I leading to discovery of novel nitrogen-enriched derivatives with enhanced anticancer profile and improved drug-like properties. J Med Chem 61: 760-776, 2018.
18. Davies JR, Jewell R, Affleck P, Anic GM, Randerson-Moor J, Ozola A, Egan KM, Elliott F, García-Casado Z, Hansson J, *et al*: Inherited variation in the PARP1 gene and survival from melanoma. Int J Cancer 135: 1625-1633, 2014.
19. Lai Y, Kong Z, Zeng T, Xu S, Duan X, Li S, Cai C, Zhao Z and Wu W: PARP1-siRNA suppresses human prostate cancer cell growth and progression. Oncol Rep 39: 1901-1909, 2018.
20. Hans CP, Zerfaoui M, Naura AS, Catling A and Boulares AH: Differential effects of PARP inhibition on vascular cell survival and ACAT-1 expression favouring atherosclerotic plaque stability. Cardiovasc Res 78: 429-439, 2008.
21. Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z: GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 45 (W1): W98-W102, 2017.
22. Minor DR, Kashani-Sabet M, Garrido M, O'Day SJ, Hamid O and Bastian BC: Sunitinib therapy for melanoma patients with KIT mutations. Clin Cancer Res 18: 1457-1463, 2012.
23. Drilon A, Siena S, Ou SI, Patel M, Ahn MJ, Lee J, Bauer TM, Farago AF, Wheler JJ, Liu SV, *et al*: Safety and antitumor activity of the multitargeted pan-TRK, ROS1, and ALK inhibitor entrectinib: Combined results from two phase I trials (ALKA-372-001 and STARTRK-1). Cancer Discov 7: 400-409, 2017.
24. Cheng Y, Wang X, Xia X, Zhang W and Tian H: A benzoxazole compound as a novel MEK inhibitor for the treatment of RAS/RAF mutant cancer. Int J Cancer 145: 586-596, 2019.
25. Erice O, Smith MP, White R, Goicoechea I, Barriuso J, Jones C, Margison GP, Acosta JC, Wellbrock C and Arozarena I: MGMT expression predicts PARP-mediated resistance to temozolomide. Mol Cancer Ther 14: 1236-1246, 2015.
26. Doedens JR and Black RA: Stimulation-induced down-regulation of tumour necrosis factor-alpha converting enzyme. J Biol Chem 275: 14598-14607, 2000.
27. Oing C, Tennstedt P, Simon R, Volquardsen J, Borgmann K, Bokemeyer C, Petersen C, Dikomey E, Rothkamm K and Mansour WY: BCL2-overexpressing prostate cancer cells rely on PARP1-dependent end-joining and are sensitive to combined PARP inhibitor and radiation therapy. Cancer Lett 423: 60-70, 2018.
28. Shen Y, Aoyagi-Scharber M and Wang B: Trapping poly(ADP-Ribose) polymerase. J Pharmacol Exp Ther 353: 446-457, 2015.
29. Raineri A, Prodomini S, Fasoli S, Gotte G and Menegazzi M: Influence of onconase in the therapeutic potential of PARP inhibitors in A375 malignant melanoma cells. Biochem Pharmacol 167: 173-181, 2019.
30. Ma X, Dang C, Min W, Diao Y, Hui W, Wang X, Dai Z, Wang X and Kang H: Downregulation of APE1 potentiates breast cancer cells to olaparib by inhibiting PARP-1 expression. Breast Cancer Res Treat 176: 109-117, 2019.
31. Alupei MC, Licarete E, Patras L and Banciu M: Liposomal simvastatin inhibits tumour growth via targeting tumour-associated macrophages-mediated oxidative stress. Cancer Lett 356: 946-952, 2015.
32. Kretzer IF, Maria DA, Guido MC, Contente TC and Maranhao RC: Simvastatin increases the antineoplastic actions of paclitaxel carried in lipid nanoemulsions in melanoma-bearing mice. Int J Nanomedicine 11: 885-904, 2016.
33. Geboers S, Stappaerts J, Tack J, Annaert P and Augustijns P: In vitro and in vivo investigation of the gastrointestinal behavior of simvastatin. Int J Pharm 510: 296-303, 2016.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.