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Original Research

Luteolin promotes the sensitivity of cisplatin in ovarian cancer by decreasing PRPA1-medicated autophagy

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Abstract: Luteolin (LUT) is a flavone universally presented in plants. It shows an anti-carcinogenic effect in different cancers and could increase the sensitivity of cisplatin in colorectal cancer cell lines through Nrf2 pathway. However, the effect of luteolin on the sensitivity to ovarian cancer cells has not been studied. In this study, luteolin was found to suppress autophagy with reduced expression of LC3-II, but enhanced the inhibition of cell vitality and promoted apoptosis induced by cisplatin, leading to restoration of the sensitivity to cisplatin in ovarian cancer cells through CCK-8, flow cytometry and immunofluorescent assays. Although cisplatin elevated the PARP1 for cell survival, the cisplatin-induced expression of PARP1 was inhibited by luteolin a dose- and time- dependent manner through Q-PCR and WB assays. Further, PARP1 siRNA could further improve the LUT-induced inhibition of cell vitality and restore the sensitivity to cisplatin with reduced LC3-II levels. Our present work demonstrate that LUT can suppresses autophagy but enhance apoptosis induced by cisplatin and promote the sensitivity to cisplatin through suppressing the expression of RARP1 in ovarian cancer.

Key words: Ovarian cancer; Luteolin; Cisplatin; Autophagy; Chemosensitivity.

Introduction

Ovarian cancer has the highest mortality rate among gynaecologic cancer. It is estimated that 22,280 new diagnoses and 14,240 deaths from this neoplasm occurred in United States in 2016 (1). Although surgery and platinum-based chemotherapy show a good therapeutic effect, about 70% patients are suffering disease recurrence and require further treatment (2,3). And recurrent and metastatic ovarian cancer often shows insensitivity to standard platinum-based chemotherapy and threatened patients' health. Thus, it is important to develop a method to improve the sensitivity of ovarian cancer to cisplatin.

Flavonoids have been widely investigated on their potent of increasing chemical sensitivity of cancer cells (4). Luteolin (LUT) is a common flavonoid found in many fruits, vegetables and herbs such as such as celery, carrot and honeysuckle. Luteolin exist in the form of glycosides and are metabolized by intestinal bacteria, cleaved and glucuronated during absorption. Recently, luteolin was reported to show cancer preventive and therapeutic function in glioblastoma, colorectal cancer and several other cancers (5-7). On ovarian cancer, luteolin is reported to enhance ovarian cancer cells to paclitaxel and down-regulate the epithelial-to-mesenchymal transition. The activities of luteolin appear to be cell type dependent in different tumorigenesis especially (8, 9). However, whether luteolin could increase the sensitivities of ovarian cancer to cisplatin has not been studied yet. Therefore, we take out this study to observe the effect of luteolin on the sensitivity of cisplatin on ovarian cancers and explore the mechanism.

Autophagy is an important survival mechanism in response to several types of stress. Previous studies have demonstrated that autophagy plays key role in various types of cancer cells in response to anticancer therapies (10). Microtubule-associated protein 1 light chain 3 (LC3)-II is a marker of autophagy and increased LC3-II levels indicate that the autophagy has been initiated. Elucidating the mechanisms involved in autophagy may provide effective strategies for cancer therapy(11). Moreover, previous studies found that PARP1 inhibition may permit a substantial lowering of cisplatin concentrations without diminishing treatment efficacy, potentially reducing systemic side effects (12, 13). More and more researchers using microRNA-PARP1, involved in cisplatin resistance in ovarian cancer, which could be regarded as a potential sensitizer in cisplatin chemotherapy (14).

PARP1 (poly(ADP-ribose) polymerase-1) is an important nuclear enzyme in repairing the single-strand damage of DNA, which can bind to DNA by two zinc finger motifs and transfer chains of ADP-ribosyl moieties/NAD+ to chromatin- associated receptor proteins (15). The inhibition of PARP in the presence of HR deficiency leads to cell death from gross genetic disarray due to a process called 'synthetic lethality' (12, 16). Previous studies found that PARP inhibitors can be used to potentiate chemotherapy and regulate cancer proli-

feration and apoptosis through inducing autophagy in different type of cancers such as nasopharyngeal carcinoma cells (17). Moreover, it is reported that PARP1 inhibition could permit a substantial lowering of cisplatin concentrations without diminishing treatment efficacy, potentially reducing systemic side effects (18).

In this study, we found that luteolin can suppress cell autophagy and improve sensitivity of ovarian cancer cells to cisplatin. Furthermore, the autophagy-related mechanism was detected and found that luteolin can reduce the expression of PARP1 and influence autophagy process in ovarian cancer, demonstrating that luteolin could improve ovarian cancer cells to cisplatin.

Materials and Methods

Cell culture, transfection and reagents.

Human ovarian cancer cell line (SKOV3 cell) was purchased from ATCC and cultured in DMEM (HyClone, USA) supplemented with 10% fetal bovine serum , (HyClone, USA) and 1% penicillin-streptomycin at 37°C with 5% CO_2. Lipofectamine 2000 (Invitrogen, USA) was used for transfecting cells according to the manufacturer's protocol. DNA plasmids or PARP1 siRNA: 5'-GAGCACUUCAUGAAAUUAUUU-3', NC: 5'-UUCUCCGAACGUGUCACGUUU-3' (Ruibo, Guangzhou, China) were mixed with Opti-MEM medium and Lipofectamine 2000 reagents before transfection. After added to the cells, the medium was replaced 6h later.

Drug treatment

The four groups in this study was described as follows: 1) no drug in the control (blank) group, 2) luteolin (20 and 40 μM) in the Icariin group, 3) Cisplatin (0, 1, 2.5, 5, 10, 20, 40, 80 μM, Sigma-Aldrich), 4) Cisplatin followed by luteolin 2 h later in the combination (Cisplatin + luteolin) group.

Quantitative real time PCR (qRT-PCR)

Total RNA was extracted using Trizol (Takara, Japan) and cDNA synthesis using Superscript II reverse transcriptase (Takara, Japan) was primed with random hexamer primers following the manufacturer's protocols. Quantitative real-time RT-PCR (qRT-PCR) was performed via $2^{-\Delta\Delta Ct}$ method to estimate relative expressions of PARP1, which were normalized to GAPDH for gene expression. The primers for PARP1 are F: 5'-CGGAGTCTTCGGATAAGCTCT-3' and R: 5'-TTTCCATCAAACATGGGCGAC -3'; and for GAPDH are F: 5'-GGAGCGAGATCCCTCCAAAAT-3' and R: 5'-GGCTGTTGTCATACTTCTCATGG-3' (Sangon, China). Real-time PCR for mRNA detection were performed using Stratagene Mx3000P Real time PCR (Agilent).

Western blotting

After transfected with the designated plasmids or siRNA, cells were harvested into universal protein extraction lysis buffer (Beyotime, China) containing protease inhibitor cocktail (Roche, Switzerland). We next measured protein concentrations and equal amounts of protein were prepared for SDS-PAGE, transferred to PVDF (Millipore, USA) and detected with primary antibody of PARP1(1:2000, ab32138, Abcam, USA) and GAPDH and horseradish peroxidase-conjugated secondary antibodies (1:10000, #51332, CST, USA). Specific proteins were visualized using an enhanced chemiluminescence (ECL, Millipore, USA) western blot detection system.

Fluorescence microscopy

Cells cultured in 6-well chamber slides were washed three times with cold PBS, fixed with 4% paraformaldehyde in PBS for 15 min, permeabilized with 0.5% Triton X-100 in PBS for 12 min. Rabbit polyclonal anti-PARP1, mouse monoclonal anti-LC3, rabbit polyclonal anti-GAPDH primary antibodies and second antibodies were purchased from Cell Signaling Technology.A stock solution of cisplatin was prepared in DMSO at 1 mg/mL and was further diluted to the final working concentrations with antibiotic-free RPMI-1640 medium. After blocking 30min with BSA, SKOV3 cells incubated with primary antibodies overnight at 4°C and followed with related-labeled secondary antibodies for 1 h at room temperature. The cells were detected with DAPI (Sigma) for nuclear staining and the images were acquired with laser scanning confocal microscope (Olympus).

Annexin V-FITC

Apoptosis was detected by translocation of phosphatidylserine to the cell surface using an Annexin V-FITC apoptosis detection kit (Beyotime, China) according to the manufacturer's protocol. Briefly, after treatment for 24 h, cells were harvested and washed twice with ice-cold PBS, then evaluated for apoptosis using a FACSCalibur flow cytometer with Annexin V-FITC. Fluorescence was observed with an excitation wavelength of 480 nm through FL-1 filter (530 nm) and FL-2 filter (585 nm).

Transmission electron microscopy

The autophagy of SKOV3 cells were evaluated by autophagosome screening under a JEM-1010 transmission electron microscope (Matsunaga Manufacturing, Co., Ltd., Gifu, Japan). SKOV3 cells were digested with 0.25% trypsin and collected in centrifuge tubes, and fixed with 2.5% glutaraldehyde, and post-fixed in 1% phosphate buffered osmium tetroxide. After being embedded, sectioned, and double-stained with uranyl acetate and lead citrate, images were captured with a transmission electron microscope (EM902A, Carl Zeiss MicroImaging GmbH, Germany).

Statistical analysis

Experiments were repeated three times. Statistical analyses were performed using SPSS version 17.0. Differences between experimental groups were determined using Students' t test or One-way ANOVA. Values of $P < 0.05$ were considered as significant and indicated by asterisks in the figures.

Results

Luteolin enhances the sensitivity of cisplatin and promoted apoptosis of ovarian cancer cells

To investigate the effect of luteolin on cisplatin sensitivity of ovarian cancer, we performed the cell proli-

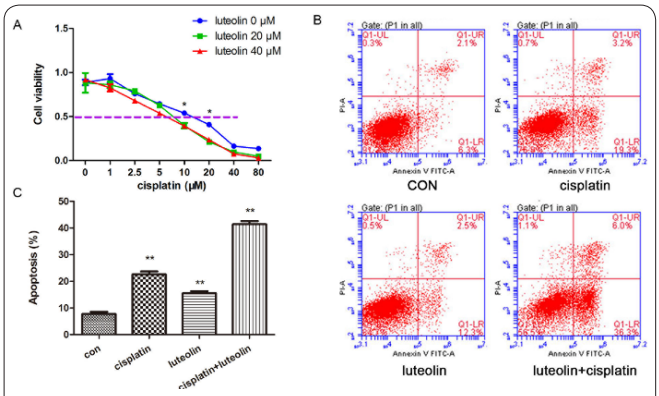

Figure 1. Luteolin promotes apoptosis and suppresses autophagy to enhance the sensitivity of cisplatin. (A) SKOV3 cells were grown in 96-well plates with treatment of different concentration of cisplatin 0, 1, 2.5, 5, 10, 20, 40, 80 uM until 50% confluent, and then treated with DMSO(control), 20uM and 40uM luteolin for 24h, respectively. The absorbance was detected at 490 nm. (B-C) Cell apoptosis were detected through flow cytometry assays with annexin V-FITC and PI staining for control, luteolin, cisplatin and luteolin+cisplatin treatment. The test was repeated three times and the image presented was typical of these three independent tests.

feration assays to administration luteolin (20, 40 μM) with cisplatin (0 - 80 μM) in ovarian cancer SKOV3 cells. The results showed that the SKOV3 cell viability obviously decreased in both added 20 μM and 40 μM luteolin group compared with control group under the high concentration of cisplatin. However, luteolin (40 μM) was more efficient than luteolin (20 μM) to enhance the cisplatin-induced inhibition of cell vitality in relative low dose of cisplatin. Thus the luteolin (40 μM) was selected for further apoptosis analysis (Fig. 1A). Then SKOV3 cells were incubated with luteolin (40 μM) and cisplatin (20 μM) and flow cytometry was followed to observe the up-regulation of cisplatin-induced apoptosis by luteolin (Fig. 1B-C). The results showed that luteolin can enhance cisplatin-induced inhibition of cell vitality and cell apoptosis.

Luteolin suppresses the autophagy of SKOV3 cells induced by cisplatin

To investigate the effect of luteolin on cisplatin sensitivity further, the autophagy of SKOV3 cells were evaluated by autophagosome screening under a transmission electron microscope to detect whether luteolin regulates this autophagy process. 3-MA was a inhibitor of autophagy and was used as positive control. The morphology of intracellular autophagosomes following treatment with cisplatin and luteolin co-treatment is shown in Fig. 2A. The results showed that luteolin further decreased autophagy induced by cisplatin. Furthermore, immunofluorescence assay for LC3-II was taken to verify the effect of luteolin on SKOV3 cells. As Fig 2B shown, compared to incubate with cisplatin alone, the expression of LC3-II in cells incubated with luteolin and cisplatin was less than that with cisplatin alone, illustrating autophagy induced by cisplatin was decreased when incubated with luteolin. Together, these results showed that luteolin can suppresses autophagy but enhance apoptosis induced by cisplatin and promote the sensitivity of cisplatin in ovarian cancer.

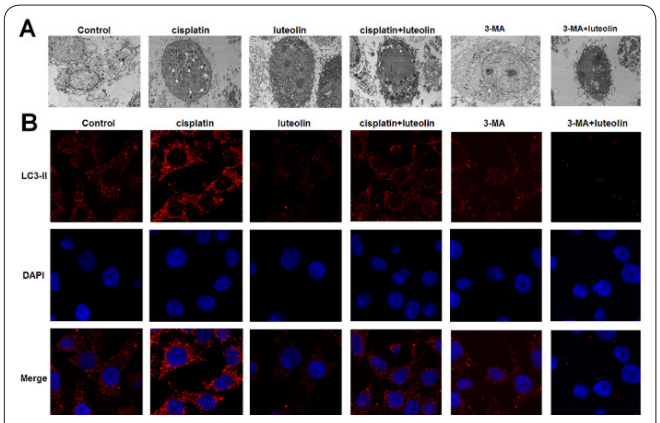

Figure 2. Luteolin suppresses autophagy induced by cisplatin. (A) With or without the treatment of autophagy inhibitor 3-MA (2 mM), electron micrographs of SKOV3 cells after for 24h incubated with cisplatin and luteolin. (B-C) With or without the treatment of autophagy inhibitor 3-MA (2 mM), SKOV3 cells were treated with luteolin and cisplatin and then observed with fluorescence microscopy to detect LC-II expression.

Luteolin suppresses the expression of PARP1 in ovarian cancer

PARP1 is a key enzyme in cell apoptosis and autophagy. To investigate the mechanism of luteolin on cisplatin sensitivity, the effect of lutoelin on PARP1 was explored. And this hypothesis was confirmed by western blot assays and RT-PCR assays. Western blot and QPCR assays were taken to observe the effect of lutoelin on PARP1. As Fig. 3A-D shown, the expression of PARP1 of SKOV3 cells treated with luteolin was shown a dose- and time- dependent manner at both protein and mRNA levels. Moreover, PARP1 and LC3-II was detected when SKOV3 treated with luteolin and cisplatin. It is shown that reveal that cisplatin can significantly increase expression of PARP1. However, when luteo-

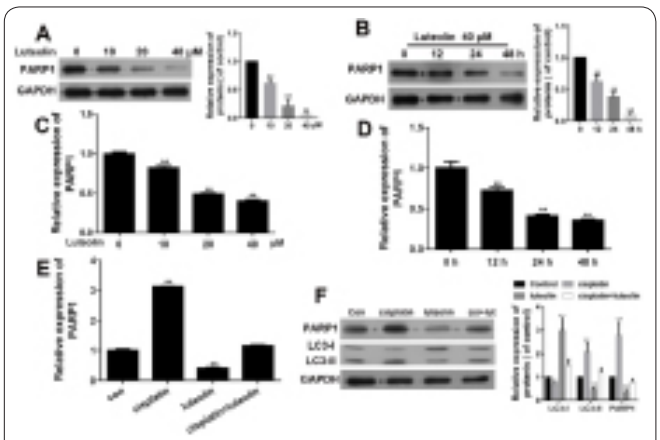

Figure 3. Luteolin suppresses the expression of PARP1 in ovarian cancer. (A) SKOV3 cells were treated with 10 uM, 20 uM and 40 uM luteolin for 24h (PBS as a bank control). Lysates were measured by Western blotting (A) and Real-time PCR (C) for PARP1. SKOV3 cells were treated with 40uM luteolin and then cultured with different time 0, 12, 24 and 28 h. Lystates were measured by Western blotting (B) and Real-time PCR (D) for PARP1. (E) RT-PCR assays reveal that cisplatin can significantly increase expression of PARP1. However, when luteolin was co-treated, cisplatin can no longer increase the expression of RARP1. (F) Western blotting assay show that cisplatin can enhance the expression of PARP1 and LC3II. But added luteolin can suppress these expression induced by cisplatin.

lin was co-treated, cisplatin can no longer increase the expression of RARP1 (Fig. 3E). It is also found that cisplatin can enhance the expression of LC3II. But added luteolin can suppress these expression induced by cisplatin (Fig. 3F). These results suggested that luteolin can decrease the expression of RARP1 which participated in autophagy induced by cisplatin.

Luteolin can promote sensitivity of cisplatin through suppressing the expression of PARP1 in ovarian cancer.

To confirm whether luteolin down-regulates the expression of PARP1 to influence cell autophagy and the sensitivity of cisplatin, PARP1 siRNA was synthesized and transfected into SKOV3 cells to knockdown the expression of PARP1. Through Western blot and QPCR assays, it is found that when PARP1 was silenced by PARP1 siRNA and the inhibitory effect of luteolin was decreased (Fig 4A-B). For the sensitivity of cisplatin, the cell proliferation assays revealed that both siPARP1 and luteolin could decrease cell viability at the same concentration of cisplatin compared with control. And the effect of luteolin on cisplatin sensitivity decreased when PARP1 siRNA was transfected (Fig. 4C). Furthermore, to detect the effect of luteolin through PARP1 pathway on SKOV3 cell autophagy, Western blot and immunofluorescence assays were taken and found that co-treatment of luteolin and siPARP1 can further decrease autophagy but the inhibitory effect of luteolin was decreased when PARP1 was transfected (Fig 4B,D). These results indicated that luteolin can promote sensitivity of cisplatin and decrease autophagy through suppressing the expression of RARP1 in ovarian cancer.

Discussion

The introduction of platinum-based drugs was landmark developments and cisplatin as the front-line chemotherapy in the treatment of ovarian cancer (19, 20). Therefore, more and more researchers have focused on enhancing the sensitivity of cisplatin to improve therapy-efficiency.

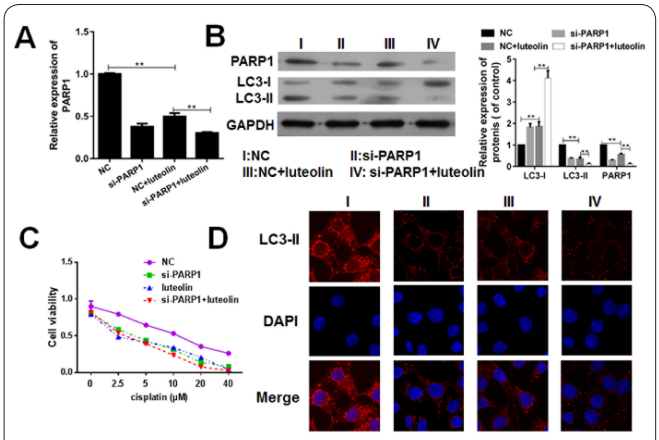

Figure 4. Luteolin can promote sensitivity of cisplatin through suppressing the expression of RARP1 in ovarian cancer. (A-B) Luteolin can enhance the knockdown-results induced by siPARP1 in SKOV3 cells. (C) Cell proliferation assays reveal SKOV3 cell viability with treatment of siPARP1, luteolin and luteolin/siPARP1 at different concentration of cisplatin. (D) Immunofluorescence assays further detect autophagy results in SKOV3 cell line through anti-LC3II antibody.

Luteolin, universally known as a constituent of vegetables and herbs, has been demonstrated to have various beneficial effects on health such as skin protection, anti-inflammatory, anti-bacterial and anti-cancer activity. Luteolin plays a key role in tumorigenesis process in several tumors but its mechanism remains largely unknown (21, 22). Our present study found that SKOV3 cell obviously decreased in luteolin group compared to control group with the same concentration of cisplatin. These results reveal that luteolin promote the sensitivity of cisplatin-therapy in ovarian cancer. Previous studies show that cisplatin is a chemotherapeutic drug mainly in part by binding to and blocking the duplication of DNA to induce cell apoptosis and autophagy (19). Further the effect of luteolin in improving the sensitivity of cisplatin in ovarian cancer was detected. The autophagy of SKOV3 cells was detected by transmission electron microscope and immunofluorescence assay and found that luteolin can increase apoptosis and decreased autophagy induced by cisplatin. Indeed, the interaction between apoptosis and autophagy was extensively investigated. On the one hand, some tumor cell enhanced the base level of autophagy to maintain mitochondrial function and energy homeostasis to meet the elevated metabolic demand of growth and proliferation. On another hand, autophagy-induced apoptosis is conceived to the treatment of cancer. Autophagic cell death is another type of cell death, which was morphologically different from apoptosis and speculated to be caused by high levels of autophagy (23, 24).

Deng et al. found that resveratrol can induce autophagy partially via activation of PARP1-SIRT1 signaling pathway (25). Chen et al. also found that PARP1 plays an important role in autophagy via AMPK/mTOR pathway in CNE2 cells (26). In recent years, it is found that PARP1 can influence the efficiency of cisplatin medicated chemotherapy and cisplatin upregulated the expression of PARP1 in Lung cancer and gastric carcinoma (27). Thus, we assume that luteolin could suppress autophagy through PARP1 pathway. Our study revealed that cisplatin can also promote the expression of PARP1 in ovarian cancer. And it is found that luteolin can inhibit the expression of PARP1 of SKOV3 cells in dose- and time- dependent manner, suggesting that PARP1 may involve in sensitizing cisplatin activity.

However, when co-treated of luteolin with cisplatin, cisplatin can no longer increase the expression of RARP1. This result shows that luteolin can regulate cisplatin medicated-PARP1 expression. Together with our previous results that luteolin can suppress the expression of PARP1 induced by cisplatin, we want to better confirm that whether luteolin down-regulates the expression of PARP1 to influence cell autophagy and the sensitivity of cisplatin-therapy. We synthesized siRNA of PARP1 and infected into SKOV3 cells to knockdown the expression of PARP1. The results showed that both siPARP1 and luteolin can decrease the expression of PARP1 at protein and RNA level. Meanwhile, we further found that luteolin can enhance the knockdown-results induced by siPARP1 when co-treatment of siPARP1 and luteolin. This laboratory finding demonstrates that luteolin can strongly decrease the expression of PARP1.

PARP1 inhibition may permit a substantial lowe-

ring of cisplatin concentrations without diminishing treatment efficacy, potentially reducing systemic side effects (13). More and more researchers using microRNA-PARP1, involved in cisplatin resistance in ovarian cancer, which could be regarded as a potential sensitizer in cisplatin chemotherapy (12). Taken together the function of PARP1 in cisplatin-related therapy at several tumors, we then speculate whether luteolin can promote sensitivity of cisplatin through suppressing the expression of RARP1 in ovarian cancer. Our results revealed that both siPARP1 and luteolin can obviously decrease cell viability at the same concentration of cisplatin compared with control. Meanwhile, there is the lowest cell viability when co-treatment of siPARP1 and luteolin from cisplatin concentration 5 μM to 40 μM. Our previous results reveal that luteolin affects the sensitivity of cisplatin through autophagy. We next detect the mechanism of this process that whether PARP1 pathway plays an important role in luteolin-medicated autophagy. Western blot assays and Immunofluorescence assays were involved and found that both luteolin and siPARP1 can decrease the expression of LC3II and suppress autophagy, respectively. Furthermore, co-treatment of luteolin and siPARP1 can further decrease autophagy. These results indicated that luteolin can promote sensitivity of cisplatin and decrease autophagy through suppressing the expression of RARP1 in ovarian cancer.

In summary, the current study reveals that luteolin can suppress autophagy but enhance apoptosis induced by cisplatin and promote the sensitivity of cisplatin through suppressing the expression of RARP1 in ovarian cancer. Our present work provides a novel insight into improve the sensitivity of cisplatin-based therapy in ovarian cancer and expands the knowledge of luteolin in tumorigenesis.

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Interest conflict

The authors declared that they have no conflict of interest.

Author's contribution

QL and YJT conceived and designed the study. QL, DCZ, BZH and ZWZ performed the experiments. QL and YJT wrote the paper.

References

1. Martin Lluesma S, Wolfer A, Harari A, Kandalaft LE. Cancer Vaccines in Ovarian Cancer: How Can We Improve? Biomedicines 2016; 4: E10.

2. Tomao F, Marchetti C, Romito A, et al. Overcoming platinum resistance in ovarian cancer treatment: from clinical practice to emerging chemical therapies. Expert Opin Pharmacother 2017; 18.

3. Verdoodt F, Kjaer SK, Friis S. Influence of aspirin and non-aspirin NSAID use on ovarian and endometrial cancer: Summary of epidemiologic evidence of cancer risk and prognosis. Maturitas 2017; 100: 1-7.

4. Hou X, Bai X, Gou X, et al. 3′,4′,5′,5,7-Pentamethoxyflavone sensitizes cisplatin-resistant A549 cells to cisplatin by inhibition of Nrf2 pathway. Mol Cells 2015; 38: 396-401.

5.Chakrabarti M, Ray SK. Anti-tumor activities of luteolin and silibinin in glioblastoma cells: overexpression of miR-7-1-3p augmented luteolin and silibinin to inhibit autophagy and induce apoptosis in glioblastoma in vivo. Apoptosis 2016; 21: 312-28.

6.Deng L, Jiang L, Lin X, Tseng KF, Lu Z, Wang X. Luteolin, a novel p90 ribosomal S6 kinase inhibitor, suppresses proliferation and migration in leukemia cells. Oncol Lett 2017; 13: 1370-8.

7.Guo YF, Xu NN, Sun W, Zhao Y, Li CY, Guo MY.Luteolin reduces inflammation in Staphylococcus aureus-induced mastitis by inhibiting NF-kB activation and MMPs expression. Oncotarget 2017; 8: 28481-93.

8.Dia VP, Pangloli P. Epithelial-to-Mesenchymal transition in paclitaxel-resistant ovarian cancer cells is downregulated by luteolin. J Cell Physiol 2017; 232: 391-401.

9. Lu DF, Yang LJ, Wang F, Zhang GL. Inhibitory effect of luteolin on estrogen biosynthesis in human ovarian granulosa cells by suppression of aromatase (CYP19). J Agric Food Chem 2012; 60: 8411-8.

10. Bravo-San Pedro JM, Kroemer G, Galluzzi L. Autophagy and mitophagy in cardiovascular disease. Circ Res 2017; 120: 1812-24.

11. Muller S, Brun S, René F, de Sèze J, Loeffler JP, Jeltsch-David H. Autophagy in neuroinflammatory diseases. Autoimmun Rev 2017; S1568-9972: 30139-8.

12. Rouleau M, Patel A, Hendzel MJ, Kaufmann SH, Poirier GG. PARP inhibition: PARP1 and beyond. Nat Rev Cancer 2010; 10: 293-301.

13. Mangerich A, Bürkle A. Pleiotropic cellular functions of PARP1 in longevity and aging: genome maintenance meets inflammation. Oxid Med Cell Longev 2012; 2012: 321653.

14. Schiewer MJ, Knudsen KE. Transcriptional roles of PARP1 in cancer. Mol Cancer Res 2014; 12: 1069-80.

15. Beck C, Robert I, Reina-San-Martin B, Schreiber V, Dantzer F. Poly(ADP-ribose) polymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. Exp Cell Res 2014; 329: 18-25.

16. Malyuchenko NV, Kotova EY, Kulaeva OI, Kirpichnikov MP, Studitskiy VM. PARP1 Inhibitors: antitumor drug design. Acta Naturae, 2015; 7: 27-37.

17. Singh SS, Sarma JA, Narasu L, Dayam R, Xu S, Neamati N. A review on PARP1 inhibitors: Pharmacophore modeling, virtual and biological screening studies to identify novel PARP1 inhibitors. Curr Top Med Chem 2014; 14: 2020-30.

18. Mangerich A, Burkle A. Pleiotropic cellular functions of PARP1 in longevity and aging: genome maintenance meets inflammation. Oxid Med Cell Longev 2012; 2012: 321653.

19. Kwon DY, Han GH, Ulak R, Ki KD, Lee JM, Lee SK. Syndrome of inappropriate antidiuretic hormone secretion following irinotecan-cisplatin administration as a treatment for recurrent ovarian clear cell carcinoma. Obstet Gynecol Sci 2017; 60: 115-7.

20. Samuel P, Pink RC, Brooks SA, Carter DR. miRNAs and ovarian cancer: a miRiad of mechanisms to induce cisplatin drug resistance. Expert Rev Anticancer Ther 2016; 16: 57-70.

21. Park SH, Park HS, Lee JH, et al. Induction of endoplasmic reticulum stress-mediated apoptosis and non-canonical autophagy by luteolin in NCI-H460 lung carcinoma cells. Food Chem Toxicol 2013; 56: 100-9.

22. Peng M, Watanabe S, Chan KWK, et al. Luteolin restricts dengue virus replication through inhibition of the proprotein convertase furin. Antiviral Res 2017; 143: 176-185.

23. Booth L A, Tavallai S, Hamed H A, et al. The role of cell signalling in the crosstalk between autophagy and apoptosis. Cellular Signalling 2014; 26:549-555.

24. Sui X, Kong N, Ye L, et al. p38 and JNK MAPK pathways

control the balance of apoptosis and autophagy in response to chemotherapeutic agents. Cancer Letters 2014; 344:174-179.
25. Wang Q, Wang H, Jia Y, Pan H, Ding H. Luteolin induces apoptosis by ROS/ER stress and mitochondrial dysfunction in gliomablastoma. Cancer Chemother Pharmacol 2017; 79: 1031-41.
26. Yan M, Liu Z, Yang H, et al. Luteolin decreases the UVA induced autophagy of human skin fibroblasts by scavenging ROS. Mol Med Rep 2016; 14: 1986-92.
27.Ji Y, Tulin AV. The roles of PARP1 in gene control and cell differentiation. Curr Opin Genet Dev 2010; 20: 512-8.