Arctigenin upregulates apoptosis through the AKT/MTOR pathway, inhibiting the proliferation of glioma

jiang yongan
Nanchang University,Jiangxi provincial People's hospital affiliated to nanchang University
Liu Jia yu
Department of neurosurgery,Peking University People's Hospital
Hong Wangwang
Nanchang University, Jiangxi provincial People's hospital affiliated to Nanchang university
Fei Xiaowei
Department of physiology,dalian Medical University
Liu ru'en (Liuruen@pku.edu.cn)
Department of Neurosurgery,peking University People's Hospital

Research article

Keywords: Arctigenin(ARG),AKT/MTOR,autophagy, apoptosis,glioma

Posted Date: December 11th, 2019

DOI: https://doi.org/10.21203/rs.2.18701/v1

Jiang yongan[1*] ,Liu jiayu[2*] ,Hong wangwang[1],Fei xiaowei[3] and Liu Ru'en[2](corresponding author)

Arctigenin upregulates apoptosis through the AKT/MTOR pathway, inhibiting the proliferation of glioma

Jiang yongan[1*]

[1]Nanchang University,Jiangxi provincial People's hospital affiliated to Nanchang University,Nanchang,Jiangxi,330006,China;

Email: [1*]1296918592@qq.com

Liu jiayu[2*]

[2]Department of Neurosurgery,Peking University People's hospital,11[th] Xizhimen South St.Beijing 100044,China;

[2*]liujiayu@pku.edu.cn

Hong wangwang[1]

[1]Nanchang University,Jiangxi provincial People's hospital affiliated to Nanchang University,Nanchang,Jiangxi,330006,China;

[1]2607251069@qq.com

Fei xiaowei[3]

[3]Department of Physiology,Dalian Medical University,Dalian,116044,China;

[3]fxwneurosurgery@sina.com

Liu Ru'en[2]

[2]Department of Neurosurgery,Peking University People's hospital,11[th] Xizhimen South St.Beijing 100044,China

Liuruen@pku.edu.cn

Correspondence to:Liu Ru'en,Department of Neurosurgery,Peking University People's hospital,11[th] Xizhimen South St.Beijing 100044. China.

Email: liuruen@pku.edu.cn

*These authors contributed equally to this work

ABSTRACT:

Arctigenin (ARG) is a natural lignan compound extracted from arctium lappa and has displayed anticancer functions and effective treatments in a variety of cancers.Studies had shown that Arctigenin(ARG) inhibits tumors through the AKT/MTOR pathway and mediates autophagy.However,the role in glioma cellshave not still fully understood.This study was designed to investigate whether Arctigenin(ARG) can mediateAKT/mTOR pathway in glioma to regulate autophagy,and affected glioma cells growth and survival.We found that the dose-dependent downregulation of Arctigenin(ARG),reducing cell proliferation,migration and invasion in two human glioblastoma cell lines (U87, T98G),These phenomena were reversed after the administration of the AKT agonist (SC79). Arctigenin(ARG) also affected other autophagy markers such as p62, LC3B.In addition, the apoptotic molecules cleaved-PARP,caspase-9, and cleaved-caspase3 were also dose-dependently altered.

KEYWORDS:Arctigenin(ARG),AKT/MTOR,autophagy, apoptosis,glioma

1. *Arctigenin upregulates apoptosis through the AKT/MTOR pathway, inhibiting the proliferation of glioma*
2. Jiang yongan[1*] ,Liu jiayu[2*] ,Hong wangwang[1],Fei xiaowei[3] and Liu Ru'en[2]
3. *Abstract*:
4. Arctigenin (ARG) is a natural lignan compound extracted from arctium lappa and has displayed
5. anticancer functions and effective treatments in a variety of cancers.Studies had shown that
6. Arctigenin(ARG) inhibits tumors through the AKT/MTOR pathway and mediates
7. autophagy.However,the role in glioma cellshave not still fully understood.This study was
8. designed to investigate whether Arctigenin(ARG) can mediateAKT/mTOR pathway in glioma to
9. regulate autophagy,and affected glioma cells growth and survival.We found that the
10. dose-dependent downregulation of Arctigenin(ARG),reducing cell proliferation,migration and
11. invasion in two human glioblastoma cell lines (U87, T98G),These phenomena were reversed after
12. the administration of the AKT agonist (SC79). Arctigenin(ARG) also affected other autophagy
13. markers such as p62, LC3B.In addition, the apoptotic molecules cleaved-PARP,caspase-9, and
14. cleaved-caspase3 were also dose-dependently altered.
15. *KEYWORDS*:Arctigenin(ARG),AKT/MTOR,autophagy, apoptosis,glioma
16. *INTRODUCTION*:

17. Glioma is the most common central nervous system tumor, and about 50% is
18. glioblastoma,Surgery, radiation therapy, and drug therapy (temozolomide) are routine ways to
19. treat Glioblastoma,But the effect of treatment is not optimistic,The overall survival rate is 12-18
20. months[1].Therefore, there is an urgent need to discover new targets or novel drugs for the
21. treatment of glioma.Arctigenin(ARG) is a bioactive lignan extracted from Arctium lappa.and has
22. various biological activities such as anti-cancer[2,3],neuroprotection[4],anti-oxidation
23. effects[5,6],anti-proliferation[7,8],anti-viral[9],and exerting a certain therapeutic effect on the
24. treatment of colorectal cancer,liver cancer,retinoblastoma, prostate cancer,It has been reported that
25. Arctigenin(ARG) can treat colorectal cancer by inducing autophagy[10].It is also reported that
26. Arctigenin(ARG) inhibited Epithelial mesenchymal transition of Hepatocellular Carcinoma and
27. by suppressing GSK3β-Dependent Wnt/β-Catenin signaling pathway[11].In addition, it has been
28. reported in the literature that Arctigenin(ARG) can inhibit the proliferation of retinoblastoma Y79
29. and also promote apoptosis[12].Although there have been previous articles stating that
30. Arctigenin(ARG) has an inhibitory effect on glioma[13,14],thus deeper mechanisms remains to be
31. discovered.While Autophagy is the process of transporting damaged, denatured or aged proteins
32. and organelles to lysosomes for digestion and degradation, which circulates and degrades
33. intracellular components in response to a lack of nutrients or growth factors to maintain
34. homeostasis. Autophagy has two mechanisms: protective mechanism and death mechanism.The
35. former protects cells from apoptosis by eliminating unfolded proteins,reducing protein load and
36. eliminating damaged organelles (mainly mitochondria),and regulating the activity of caspase8 to
37. regulate cells.Apoptosis, it has been found in the literature that ATG inhibitors can greatly
38. increase caspase8 to cause hepatocyte apoptosis induced by LPS and GalN.The latter is thought to
39. be caused by complete degradation of cellular components due to excessive autophagy,manifested
40. by caspase-8 activation and the formation of apoptotic intracellular death-inducing signaling
41. complex (iDISC),such as knockdown of ATG5, which inhibits ATG7 The initial phase of

phagocytosis to reduce the production of caspase-8 and the occurrence of apoptosis,there are also articles that the degradation of caspase-8 can protect U87 glioma cells from H2O2 injury.However,the AKT/mTOR pathway plays an important role in a variety of cancers,except that it is involved in the differentiation of bone marrow mesenchymal stem cells and various osteoblasts,mainly mediated by autophagy.In this study, we have investigated the effects of Arctigenin(ARG) on the cycle and apoptosis of glioma cells (U87, T98) and further explored whether these effects are due to the AKT/mTOR pathway.In addition,we further determined the important role played by autophagy.

Methods and Materials

Chemicals,Reagents and Antibodies

ARG was purchased from tianjing wanxiang Science and Technology Ltd (Tianjin, China).ARG was dissolved in Dimethyl sulfoxide (DMSO) and diluted to 10 mM with phosphate buffered solution (PBS) and stored at 4℃.Dulbecco's modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were (Cambridge,MA).purchased from Gibco(GrandIsland,USA),Antibodies against AKT,P-AKT,AMPK,P- AMPK,mTOR,P-mTOR,p62,LC3B was purchased from Abcam.

Cell Culture

The glioma cell lines(U87,T98) was purchased from Chinese Academy of Medical Sciences (Beijing, China).and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (FBS) and Incubated in an incubator containing 5% CO2 at 37 °C.

Cell Viability Assay

2 × 103 cells were uniformly cultured in 96-well plates for 0h, 12h, 24h, 48h, 60h, 72h, and treated with different concentrations of ARG. Add 10μl of CCK8 to each empty and continue to incubate at 37 °C for 30min, The solution was detected by the microplate reader.

Cell monoclonal formation assay

Approximately 5000 cells were cultured in a 10 cm culture dish,and the cells were treated with ARG at a concentration of 100μM.200μM.400μM for 15 days.After the treatment,the cells were washed 2-3 times with PBS,fixed with 4% paraformaldehyde for 15 min,and fixed with crystal violet.And calculate the number under the microscope.

Cell Apoptosis Analysis

The mixture was thoroughly mixed in 500 μl of binding buffer,5 μl of propidium iodide (PI),and 5 μl of FITC-conjugated anti-Annexin V antibody,and the results were measured by Accui C6 flow cytometer (BD, USA) within one hour.

Cell Cycle Analysis

1 × 105 cells (U87, T98) were incubated overnight in 6-well plates, and cells were treated with different concentrations of ARG.The cells were collected, washed with PBS for 2-3 times, resuspended in 70% ethanol for 24 h, and then washed with ethanol in PBS, and added to the mixture with PI/RNase Staining Buffer. Finally,Accui C6 flow cytometer (BD,USA)) was used to measure results.

Western Blotting

Cell lysates of glioma cells (U87, T98) were used, and proteins were extracted using Pro-prepTM protein extract (Korea iNtRON Biotechnology).Proteins were separated on different concentrations of sulfate-polyacrylamidegel electrophoresis (SDS-PAGE) and transferred to

polyvinylidene difluoride (PVDF) membranes (Merck,KGaA,Darmstadt,Germany).The membrane was blocked with 5% bovine serum albumin(BSA)for 1.5 h at room temperature and incubated overnight at 4 °C with the diluted primary antibody.After incubating the secondary antibody conjugated with HRP were incubated for 1 h at room temperature, the results were obtained with electchemiluminescence(ECL)(Pierce,Rockford, IL, USA).

Statistical Analysis

The experiment was repeated at least three times and using an independent t test to compare between the two groups.One-way analysis of variance was used to compare analysis between multiple groups on SPSS 20.0 software.$P < 0.05$ was considered statistically significant.

RESULT

ARG Inhibits The Growth Of Glioblastoma Cells

To investigate whether ARG had an effect on glioma cells,we applied CCK8 assay to detect the cell vability of U87MG and T98G,These Glioma cells were treated with different concentrations of ARG in U87MG and T98G, cell activity decreased from 60% to 40% when the ARG concentration was reduced from 200 μM to 400μM at 48h(Figure,1a),In the next experiment, we used 100μM,200μM,400)μM for following experiments.and we functioned the monoclonal formation assay to verify the anchorage-independent growth of Glioblastoma cells(Figure,1b).In the ARG-treated U87MG,colony formation was reduced by 80% compared with the untreated group.the Edu assay revealed that the ability of ARG to inhibit glioma is concentration dependent.The inhibition rate exceeded 70% at a concentration of 400μM (Figure,1c).To determine the cause and mechanism of ARG inhibition of glioma cell proliferation, we used PI/rNase staining buffer to assess the glioma cell cycle progression after treatment.The toxic effects of ARG on glioma cells (U87MG, T98G) via G1/S phase arrested and induced apoptosis,The results showed that the ratio of G1/S cells of U87MG and T98G treated with ARG increased significantly.And significantly inhibited the proportion of cells in G2/M phase at a concentration of 400μM (Figure,1d); further use PI-FITC-annexin to determine the effect of ARG on apoptosis of glioma cells,as shown in the (figure,1.e),ARG dose-dependent glioma cells (U87MG, T98G),inducing early apoptosis and late apoptosis,In the Figure,we observed early apoptosis induced by ARG at 100μM,and in the transition from 200μM to 400μM,early apoptosis turned into late apoptosis,increasing from 0.5% to 2.3%.The increase in dead cells is not obvious.We also used Western blotting to determine the expression levels of the relevant CDK4,Cyclin D1,the results (Figure 2b) owed that ARG can inhibit the protein expression of CDK4 and Cyclin D1 to a greater extent.In addition,such as caspase-9,cleaved-caspase-3,bad,bax protein,ARG can significantly affect their expression after treatment of glioma cells(Figure 2b).Therefore,we confirmed that ARG induced cell cycle arrested and apoptosis.

Fig.1:Effect of Arctigenin on proliferation,cell cycle and apoptosis in U87MG and T98G. ***a.***Cell was seeding in 96-wells plates and were treated with indicated concentrations of PF for hours by CCK-8 assay.***b.***5000 cells were incubated with 100 μM,200 μM,400 μM ARG for 15 days,and It was fixed with 4% paraformaldehyde for 15 min and stained with crystal violet for 1 h.***c.***Cells were incubated with 200 μM,400 μM ARG for 48 hours,and cell proliferation using Edu asssy,The nucleus used DAPI(blue),and the proliferated cells used Azide 488(green).***d.***Apoptosis was detected by PI-FITC-annexin assay and measured within 1hours,The Annexin V-FITC axis represents cells that are early apoptotic,and the PI axis represented cells in the mid-late stage of apoptosis.**e.**The cell cycle was determined by PI/rNase staining buffer assay, and the results were

measured with an Accui C6 flow cytometer after 15 min. All experiments were repeated three times,*p<0.05;**p<0.01;***P<0.001.

ARG regulated AKT/mTOR and levels of autophagy-associated proteins

Many reports that the AKT/mTOR pathway plays an important role in tumors.We therefore considered whether the AKT/mTOR pathway is relevant in the mechanism of ARG treatment of glioblastoma.After treatment with ARG at a concentration of 100 μM, 200 μM, 400 μM for 48 h,treated glioma cells(U87MG, T98G) significantly reduced the expression of phosphorylated AKT, mTOR,correspondingly regulated the expression of phosphorylated akt,mTOR.but there were no significant changes in AKT, mTOR, and AMPK of total protein level(Figure 2a).Our study of ARG may inhibit glioma cell proliferation by AKT/mTOR-mediated autophagy pathway.Therefore,we further used Western blotting to reveal the expression of important autophagy-associated marker factors (Figure,2a).The cells were treated at a concentration of 100 μM,200 μM,and 400 μM for 48 h.It is clear that ARG can significantly increase the expression of LC3B II and P62 expression.Therefore,we believe that the reason ARG affects glioma cell proliferation may be due to AKT/mTOR-mediated autophagy.

Fig.2.ARG regulated AKT/mTOR and levels of autophagy-associated proteins ***a.***Western blotting was used to show AKT,P-AKT,mTOR,P-mTOR,LC3B,P62 expression levels on ARG-treated U87MG,T98G.***b.***Western blotting was applied to display protein expression levels on ARG-treated U87MG,T98G cell cycle and apoptosis.All experiments were repeated three times,*p<0.05;**p<0.01;***P<0.001.

SC79 significantly rescued proliferation,cell cycle arrested,apotosis induced by ARG in glioma cells

It was previously demonstrated that ARG may induce autophagy by AKT/mTOR to inhibit proliferation of glioma cells and affect cell cycle arrested and apoptosis.To further confirm,we used an agonist, SC79, an activator of AKT, to restore the ability of the AKT pathway.We found that glioma cells U87MG,T98G,were incubated in ARG and SC79 for 48 hours.The proliferative capacity was tested by Edu assay,and it was found that the group treated with ARG and SC79 was more significant than the group treated with ARG alone(Figure,3a). The result indicated ARG significantly restore the proliferation of glioma cells,and we next applyed PI/rNase staining buffer,PI-FITC-annexin to determine their cycle and apoptosis.Interestingly,SC79 not only restores their proliferative capacity,but also repairs cycle arrest and increased apoptosis(Figure,3b),The expression of cell cycle-associated proteins and apoptosis-related proteins was verified by Western blotting(Figure 4b).The results showed that SC79 can reverse the toxic effects of ARG on glioma cells.

Fig,3**:** SC79 regulated glioma cell proliferation inhibition, cycle arrested, and autophagy apoptosis treated with ARG.***a***.After 48 hours of incubation with ARG (400μM) and SC79(5μg/μL),nuclei was stained with DAPI.Edu assay was applied to detection and observation was performed using a confocal microscope***.b.***After 48 hours,the treated cells were collected and fixed with 70% ethanol for 12 hours, centrifuged and washed twice with PBS,treated with PI/rNase staining buffer assay,and detected by Accui C6 flow cytometer after 15 minutes.**c.**After 48 hours, the suspended treated cells were collected, and 20,000 cells were counted.After treatment with PI-FITC-annexin assay,the results were measured with a C6 flow cytometer within 1 hours.All experiments were repeated three times,*p<0.05;**p<0.01;***P<0.001.

SC79 reversed autophagy of glioma cells induced by ARG through AKT/mTOR pathway

As demonstrated by previous experiments,SC79 can reverse the growth inhibition of glioma caused by ARG and can reverse its effect on cycle and apoptosis.To this end,we further study whether SC79 also causes the above phenomenon through the AKT/mTOR pathway.Western blotting technology is applied to next explorations. As shown in the (figure.4 a), after administratored to the AKT agonist SC79,the proteins associated with the AKT/mTOR pathway are altered,and the phosphorylated proteins of AKT and mTOR are quite different from those of ARG alone, such as down-regulating P- The expression level of AKT,while total AKT did not change significantly,and phosphorylated mTOR also showed the same result.So far,we have further determined the toxic effects of ARG on glioma cells (U87MG, T98G),and this inhibition is mediated by the AKT/mTOR pathway,and SC79 can reverse this result. Interestingly,SC79 also reversed the reduction of autophagy-associated molecules (LC3B II) in glioma cells caused by ARG and reduced the conversion of LC3B I to LC3B II.Therefore,we suspect that the target of ARG inhibition of glioma may be related to autophagy, and further experimental evidence is needed.

Figure,4**:** SC79 regulated autophagy of glioma cells induced by ARG through AKT/mTOR pathway**.a.** Proteins were separated on different concentrations of SDS-PAGE and incubation with the corresponding monoclonal antibody for more than 18 hours, secondary antibody incubation for less than 2 hours,the results were obtained with ECL,was analyzed with the Bio-Rad gel imaging system.**b.** The detection method was the same according to the previous Western blot technique, the corresponding primary antibody was used, and the results were observed with the Bio-Rad gel imaging system.All experiments were repeated three times,*p<0.05;**p<0.01;***P<0.001.

DISSCUSSION

As the main source of therapeutic drugs,plant extracts are increasingly showing that extracts from plants can greatly prolong the survival of patients and are potential medical treasures in the future. As an extract of Arctium lappa,Arctigenin (ARG) has important potential for anti-cancer,and there are reports that it also has therapeutic effects in the treatment of cancer[10,15,16,6].However, in our current study, we further determined that Arctigenin (ARG) mediates autophagy via the AKT/mTOR pathway to inhibit glioma proliferation,cell cycle arrested, and apoptosis.Our aim was to investigate the treatment of ARG in Glioblastoma.However,ARG also inhibits the survival of hepatocellular carcinoma,inhibits cell proliferation induced by ROS-mediated mitogen-activated protein kinases[17,18],and inhibits epithelial-mesenchymal transition in peritoneal mesenchymal cells[19]. ARG-enhanced cisplatin has also been reported,incresasing sensitivity to drug resistance in colorectal cancer cells[10].Therefore,in order to study the mechanism of ARG treatment of glioma,further to determine its potential therapeutic targets.In the present study,ARG dose-dependently inhibited the proliferation of glioma cells,and the monoclonal ability of glioma cells was also inhibited by ARG.Abnormal changes in the activity of the cell cycle and apoptosis-related proteins will result in the proliferation of the cells themselves.Cell cycle regulators are therefore also considered to be very attractive targets for the treatment of tumors.In our study, we revealed that ARG can block cell cycle arrest caused by G1/S arrested.We also detected dose-dependent expression of cytosin CDK4 and Cyclin D1 in U87MG,T98G after treatment with ARG.However,there are articles pointing out that ARG blocks glioma cell cycle in G0 phase[13].At the same time, apoptosis is also considered to be the mechanism of programmed cell death and is considered to be an important and essential

therapeutic and selecttive target for the treatment of tumors.ARG has been confirmed by various studies to play a role in inducing apoptosis in different cancers.For example,in hepatocellular carcinoma,colorectal cancer,retinoblastoma[20,12],lymphoma[21,22].ARG can be triggered by intracellular signals by different pathways, such as apoptosis, such as genotoxic stress, or by external signals, such as binding of ligands to cell surface death receptors[23-25].Our research shows that ARG was appied to induce the apoptosis of glioma (U87MG, T98G),And a concentration-dependent transition of early apoptosis to late apoptosis ,futher deplored expression levels of related apoptosis proteins caspase-9, cleaved-caspase-3 were increased.After we used the AKT activator SC79, the cell cycle arrested and Apoptosis was removed.Autophagy, another mechanism of death, has the basic function of degrading cellular proteins and organelle fragments to recover new nutrients, maintaining cellular homeostasis of normal physiological functions. It has been previously reported in the literature that ARG activation posture enhances the sensitivity of cisplatin to drug-resistant colorectal cancer. The microtubule-associated protein LC3 is an important autophagic regulator, and the conversion of LC3B I to LC3B II is considered to be the mechanism of autophagy. It has cytoprotective and cytotoxic effects.In our study,we determined by Western blotting that ARG dose-dependent increases the expression of LC3B II and P62 in glioma cells.This is a classic manifestation of autophagy. Therefore, ARG is considered as an important regulator of the treatment of glioma and induction of autophagy. However,mTOR is considered as a key protein regulating autophagy,and it has been reported in the literature that autophagy regulates the progression of gastric cancer[20,26],pancreatic cancer[27-29],and esophageal cancer[30,31].The role of AKT in the proliferation of tumors cannot be ignored, by activating mTOR phosphorylation to the occurrence of autophagy.It acts as an intermediate medium between upstream protein kinase B (Akt) and downstream p70S6K and 4EBP1.We used Western blotting to detect AKT, mTOR and phosphorylated forms.ARG alone reduced the level of phosphorylated protein in a dose-dependent manner, whereas after co-treatment with AKT agonist SC79,the phosphorylated form of AKT,mTOR,P70S6K increased.Our studies indicate that ARG leads to activation of autophagy via the AKT/mTOR pathway.This indicates that ARG inhibits glioma cell proliferation by activating autophagy by AKT/mTOR.Although we have perfected the in vitro experiments,we have not been able to determine the role of ARG in the body because we have not performed in vivo experiments,so further proof is needed.In summary,Arctigenin (ARG) inhibits proliferation of glioblastoma,inhibits cell cycle,and increases apoptosis.This mechanism involves AKT/mTOR signaling.Our studies also confirm that Arctigenin (ARG) can be used as a potential promising treatment for glioblastoma.

Conflict of interest: The authors report no conflicts of interest in this work.

Funding:This work was supported by grant from the “National Natural Science Foundation of China” (Grant No. 81573774).

Ethical approval:This research does not include any studies with human participants or animals by any author.

Informed consent: This work received consent from all individuals involved in the research.

References

1. Lim M, Xia Y, Bettegowda C, Weller M (2018) Current state of immunotherapy for glioblastoma. Nat Rev Clin Oncol 15 (7):422-442. doi:10.1038/s41571-018-0003-5

2. Awale S, Lu J, Kalauni SK, Kurashima Y, Tezuka Y, Kadota S, Esumi H (2006) Identification of arctigenin as an antitumor agent having the ability to eliminate the tolerance of cancer cells to nutrient starvation. Cancer Res 66 (3):1751-1757. doi:10.1158/0008-5472.CAN-05-3143
3. Lee J, Imm JY, Lee SH (2017) beta-Catenin Mediates Anti-adipogenic and Anticancer Effects of Arctigenin in Preadipocytes and Breast Cancer Cells. J Agric Food Chem 65 (12):2513-2520. doi:10.1021/acs.jafc.7b00112
4. Yi JM, Shin S, Kim NS, Bang OS (2019) Neuroprotective Effects of an Aqueous Extract of Forsythia viridissima and Its Major Constituents on Oxaliplatin-Induced Peripheral Neuropathy. Molecules 24 (6). doi:10.3390/molecules24061177
5. Yao X, Zhu F, Zhao Z, Liu C, Luo L, Yin Z (2011) Arctigenin enhances chemosensitivity of cancer cells to cisplatin through inhibition of the STAT3 signaling pathway. Journal of cellular biochemistry 112 (10):2837-2849. doi:10.1002/jcb.23198
6. Zhang WZ, Jiang ZK, He BX, Liu XB (2015) Arctigenin Protects against Lipopolysaccharide-Induced Pulmonary Oxidative Stress and Inflammation in a Mouse Model via Suppression of MAPK, HO-1, and iNOS Signaling. Inflammation 38 (4):1406-1414. doi:10.1007/s10753-015-0115-3
7. Cho JY, Kim AR, Yoo ES, Baik KU, Park MH (1999) Immunomodulatory effect of arctigenin, a lignan compound, on tumour necrosis factor-alpha and nitric oxide production, and lymphocyte proliferation. The Journal of pharmacy and pharmacology 51 (11):1267-1273. doi:10.1211/0022357991777001
8. Li A, Wang J, Wu M, Zhang X, Zhang H (2015) The inhibition of activated hepatic stellate cells proliferation by arctigenin through G0/G1 phase cell cycle arrest: persistent p27(Kip1) induction by interfering with PI3K/Akt/FOXO3a signaling pathway. European journal of pharmacology 747:71-87. doi:10.1016/j.ejphar.2014.11.040
9. Hu Y, Chen WC, Shen YF, Zhu B, Wang GX (2019) Synthesis and antiviral activity of a new arctigenin derivative against IHNV in vitro and in vivo. Fish Shellfish Immunol 92:736-745. doi:10.1016/j.fsi.2019.07.006
10. Wang Y, Lina L, Xu L, Yang Z, Qian Z, Zhou J, Suoni L (2019) Arctigenin enhances the sensitivity of cisplatin resistant colorectal cancer cell by activating autophagy. Biochem Biophys Res Commun 520 (1):20-26. doi:10.1016/j.bbrc.2019.09.086
11. Lu Z, Chang L, Zhou H, Liu X, Li Y, Mi T, Tong D (2019) Arctigenin Attenuates Tumor Metastasis Through Inhibiting Epithelial-Mesenchymal Transition in Hepatocellular Carcinoma Suppressing GSK3β-Dependent Wnt/β-Catenin Signaling Pathway and. Frontiers in pharmacology 10:937. doi:10.3389/fphar.2019.00937
12. Ke N, Liu Q, Pi L, Fang J, Chen L, Chen X (2019) The antitumor function of arctigenin in human retinoblastoma cells is mediated by jagged-1. Molecular medicine reports 19 (5):3642-3648. doi:10.3892/mmr.2019.10026
13. Maimaitili A, Shu Z, Cheng X, Kaheerman K, Sikandeer A, Li W (2017) Arctigenin, a natural lignan compound, induces G0/G1 cell cycle arrest and apoptosis in human glioma cells. Oncol Lett 13 (2):1007-1013. doi:10.3892/ol.2016.5474
14. Zater H, Huet J, Fontaine V, Benayache S, Stevigny C, Duez P, Benayache F (2016) Chemical constituents, cytotoxic, antifungal and antimicrobial properties of Centaurea diluta Ait. subsp. algeriensis (Coss. & Dur.) Maire. Asian Pac J Trop Med 9 (6):554-561. doi:10.1016/j.apjtm.2016.04.016
15. Yoon SB, Park HR (2019) Arctigenin Inhibits Etoposide Resistance in HT-29 Colon Cancer Cells during Microenvironmental Stress. J Microbiol Biotechnol 29 (4):571-576. doi:10.4014/jmb.1901.01061

16. Zhang M, Cai S, Zuo B, Gong W, Tang Z, Zhou D, Weng M, Qin Y, Wang S, Liu J, Ma F, Quan Z (2017) Arctigenin induced gallbladder cancer senescence through modulating epidermal growth factor receptor pathway. Tumour Biol 39 (5):1010428317698359. doi:10.1177/1010428317698359
17. Lu Z, Zhou H, Zhang S, Dai W, Zhang Y, Hong L, Chen F, Cao J (2019) Activation of reactive oxygen species-mediated mitogen-activated protein kinases pathway regulates both extrinsic and intrinsic apoptosis induced by arctigenin in Hep G2. J Pharm Pharmacol. doi:10.1111/jphp.13180
18. Zhang E, Wang X, Liu X, Liang M, Hu H, Yin S (2019) 3'-Desmethylarctigenin induces G2/M cell cycle arrest and apoptosis through reactive oxygen species generation in hepatocarcinoma cells. Phytother Res. doi:10.1002/ptr.6496
19. Jin G, Su Y, Dong Q, Zhao X, Zhang L, Yan X (2019) Arctigenin alleviates TGF-beta1-induced epithelial-mesenchymal transition and PAI-1 expression via AMPK/NF-kappaB pathway in peritoneal mesothelial cells. Biochem Biophys Res Commun 520 (2):413-419. doi:10.1016/j.bbrc.2019.09.130
20. Jeong JB, Hong SC, Jeong HJ, Koo JS (2011) Arctigenin induces cell cycle arrest by blocking the phosphorylation of Rb via the modulation of cell cycle regulatory proteins in human gastric cancer cells. Int Immunopharmacol 11 (10):1573-1577. doi:10.1016/j.intimp.2011.05.016
21. Dombradi GA (1970) Tumour-growth inhibiting substances of plant origin. II. The experimental animal tumour-pharmacology of arctigenin-mustard. Chemotherapy 15 (4):250-265. doi:10.1159/000220689
22. Su S, Cheng X, Wink M (2015) Cytotoxicity of arctigenin and matairesinol against the T-cell lymphoma cell line CCRF-CEM. J Pharm Pharmacol 67 (9):1316-1323. doi:10.1111/jphp.12426
23. Ahmed B, Ali Ashfaq U, Usman Mirza M (2018) Medicinal plant phytochemicals and their inhibitory activities against pancreatic lipase: molecular docking combined with molecular dynamics simulation approach. Nat Prod Res 32 (10):1123-1129. doi:10.1080/14786419.2017.1320786
24. Jin JS, Lee JH, Hattori M (2013) Ligand binding affinities of arctigenin and its demethylated metabolites to estrogen receptor alpha. Molecules 18 (1):1122-1127. doi:10.3390/molecules18011122
25. Ogungbe IV, Crouch RA, Demeritte T (2014) (-) Arctigenin and (+) pinoresinol are antagonists of the human thyroid hormone receptor beta. J Chem Inf Model 54 (11):3051-3055. doi:10.1021/ci500537e
26. Li H, He C, Wang X, Wang H, Nan G, Fang L (2019) MicroRNA-183 affects the development of gastric cancer by regulating autophagy via MALAT1-miR-183-SIRT1 axis and PI3K/AKT/mTOR signals. Artif Cells Nanomed Biotechnol 47 (1):3163-3171. doi:10.1080/21691401.2019.1642903
27. Singh BN, Kumar D, Shankar S, Srivastava RK (2012) Rottlerin induces autophagy which leads to apoptotic cell death through inhibition of PI3K/Akt/mTOR pathway in human pancreatic cancer stem cells. Biochem Pharmacol 84 (9):1154-1163. doi:10.1016/j.bcp.2012.08.007
28. Vitale G, Zappavigna S, Marra M, Dicitore A, Meschini S, Condello M, Arancia G, Castiglioni S, Maroni P, Bendinelli P, Piccoletti R, van Koetsveld PM, Cavagnini F, Budillon A, Abbruzzese A, Hofland LJ, Caraglia M (2012) The PPAR-gamma agonist troglitazone antagonizes survival pathways induced by STAT-3 in recombinant interferon-beta treated pancreatic cancer cells. Biotechnol Adv 30 (1):169-184. doi:10.1016/j.biotechadv.2011.08.001
29. Yu CC, Chiang PC, Lu PH, Kuo MT, Wen WC, Chen P, Guh JH (2012) Antroquinonol, a natural ubiquinone derivative, induces a cross talk between apoptosis, autophagy and senescence in human pancreatic carcinoma cells. J Nutr Biochem 23 (8):900-907. doi:10.1016/j.jnutbio.2011.04.015

30. Feng J, Qi B, Guo L, Chen LY, Wei XF, Liu YZ, Zhao BS (2017) miR-382 functions as a tumor suppressor against esophageal squamous cell carcinoma. World J Gastroenterol 23 (23):4243-4251. doi:10.3748/wjg.v23.i23.4243
31. Wu N, Zhu Y, Xu X, Zhu Y, Song Y, Pang L, Chen Z (2018) The anti-tumor effects of dual PI3K/mTOR inhibitor BEZ235 and histone deacetylase inhibitor Trichostatin A on inducing autophagy in esophageal squamous cell carcinoma. J Cancer 9 (6):987-997. doi:10.7150/jca.22861

Figures

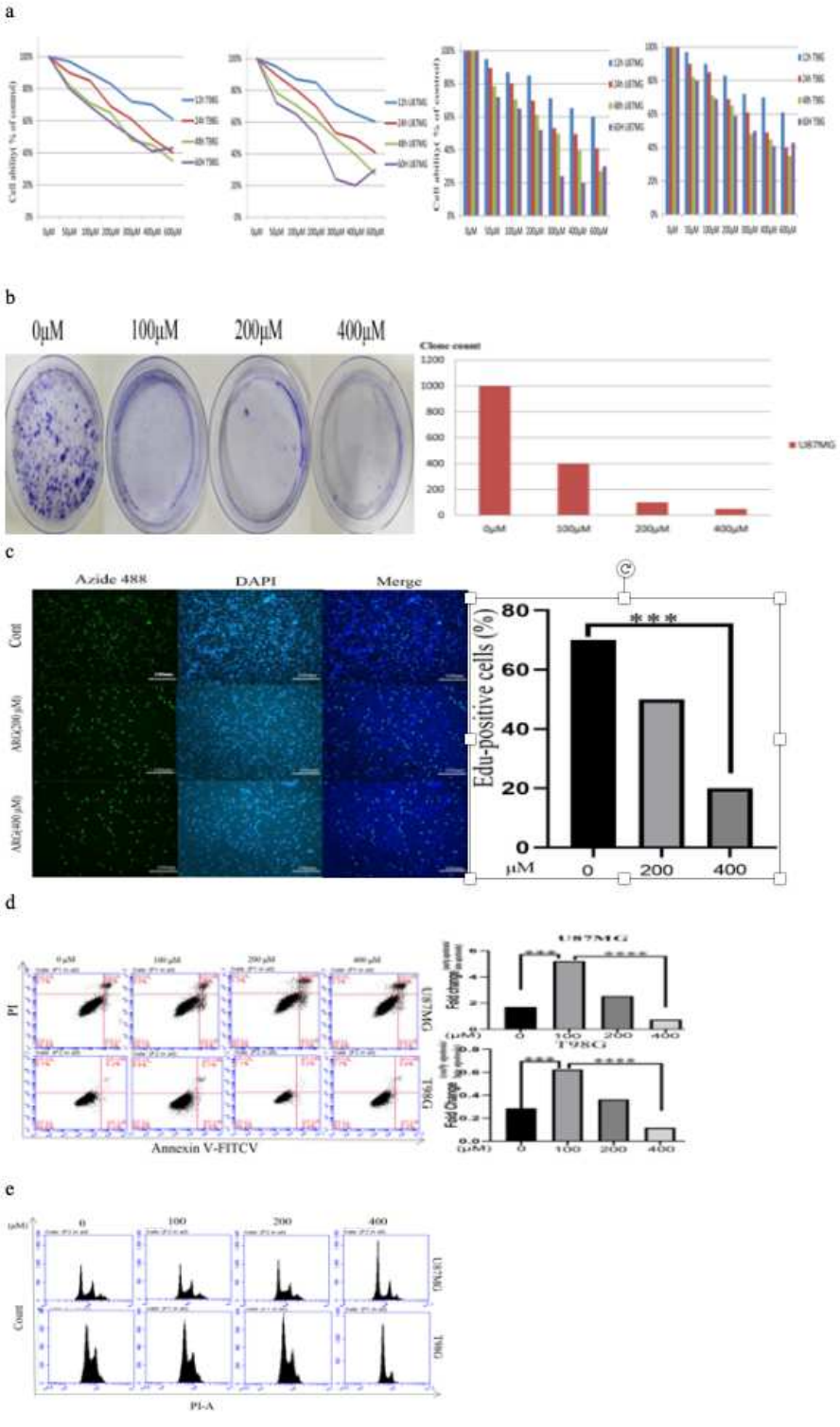

Figure 1

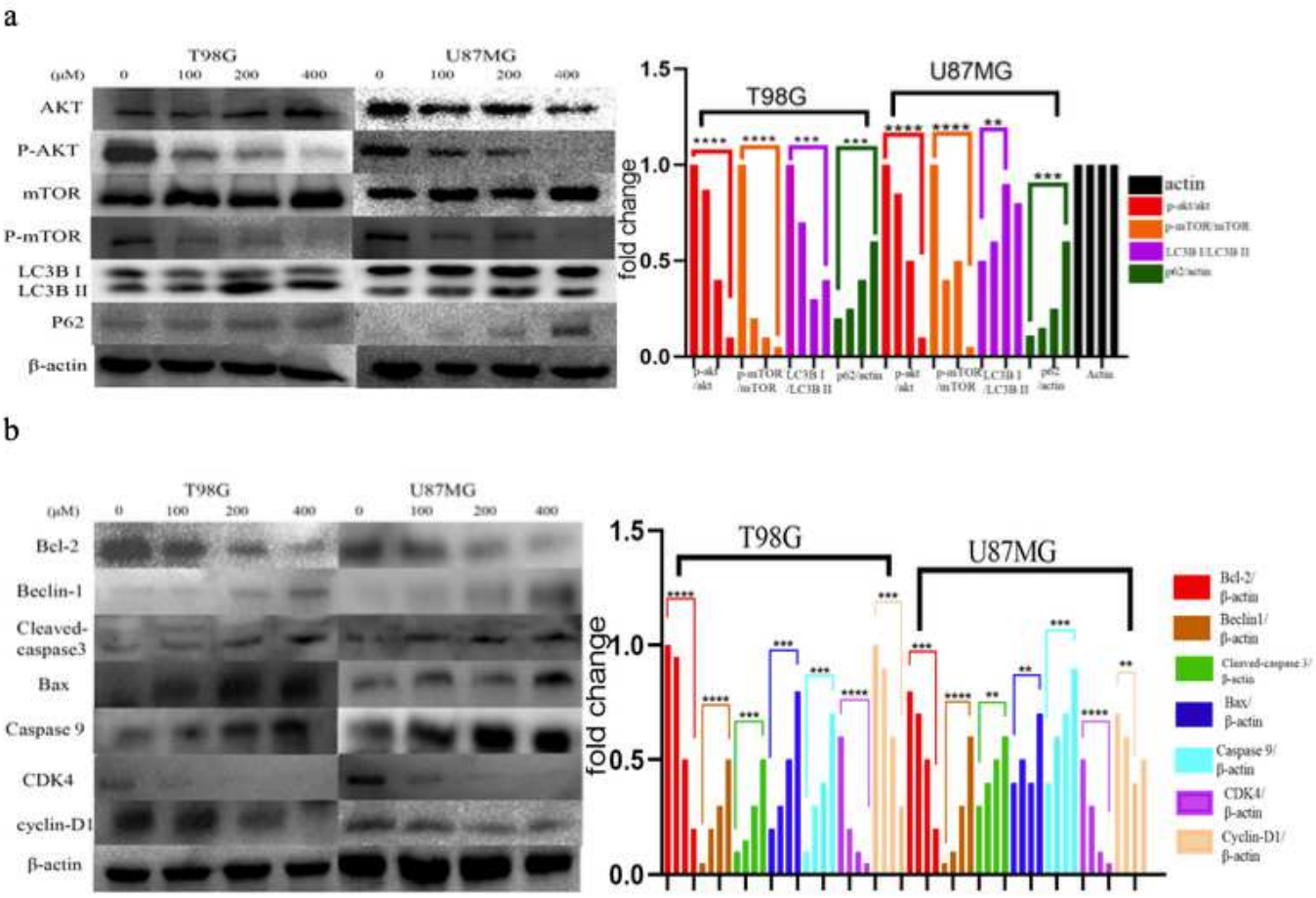
a
T98G
U87MG
(μM) 0 100 200 400 0 100 200 400
AKT
P-AKT
mTOR
P-mTOR
LC3B I
LC3B II
P62
β-actin
fold change
1.5
1.0
0.5
0.0
T98G
U87MG
actin
b
T98G
U87MG
(μM) 0 100 200 400 0 100 200 400
Bcl-2
Beclin-1
Cleaved-caspase3
Bax
Caspase 9
CDK4
cyclin-D1
β-actin
fold change
1.5
1.0
0.5
0.0
T98G
U87MG
Bcl-2/β-actin
Beclin1/β-actin
Cleaved-caspase 3/β-actin
Bax/β-actin
Caspase 9/β-actin
CDK4/β-actin
Cyclin-D1/β-actin

Figure 2

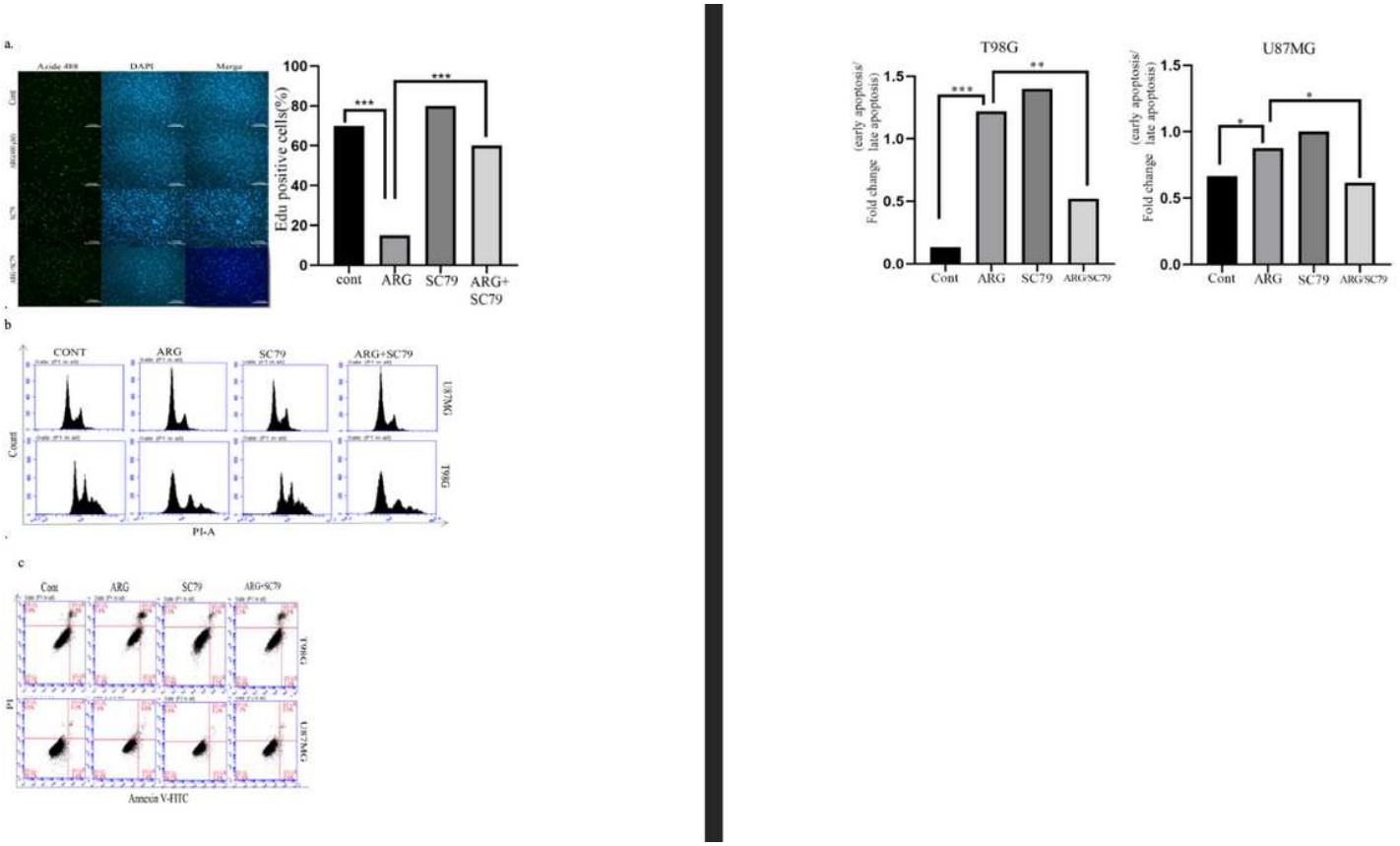
a.
Azide 488
DAPI
Merge
Cont
ARG
SC79
ARG+SC79
Edu positive cells(%)
100
80
60
40
20
0
***
***
cont
ARG
SC79
ARG+ SC79
b
CONT
ARG
SC79
ARG+SC79
U87MG
T98G
Count
PI-A
c
Cont
ARG
SC79
ARG+SC79
T98G
U87MG
PI
Annexin V-FITC
T98G
U87MG
(early apoptosis/ late apoptosis)
Fold change
1.5
1.0
0.5
0.0
***
**
Cont
ARG
SC79
ARG+SC79
(early apoptosis/ late apoptosis)
Fold change
1.5
1.0
0.5
0.0
*
*
Cont
ARG
SC79
ARG+SC79

Figure 3

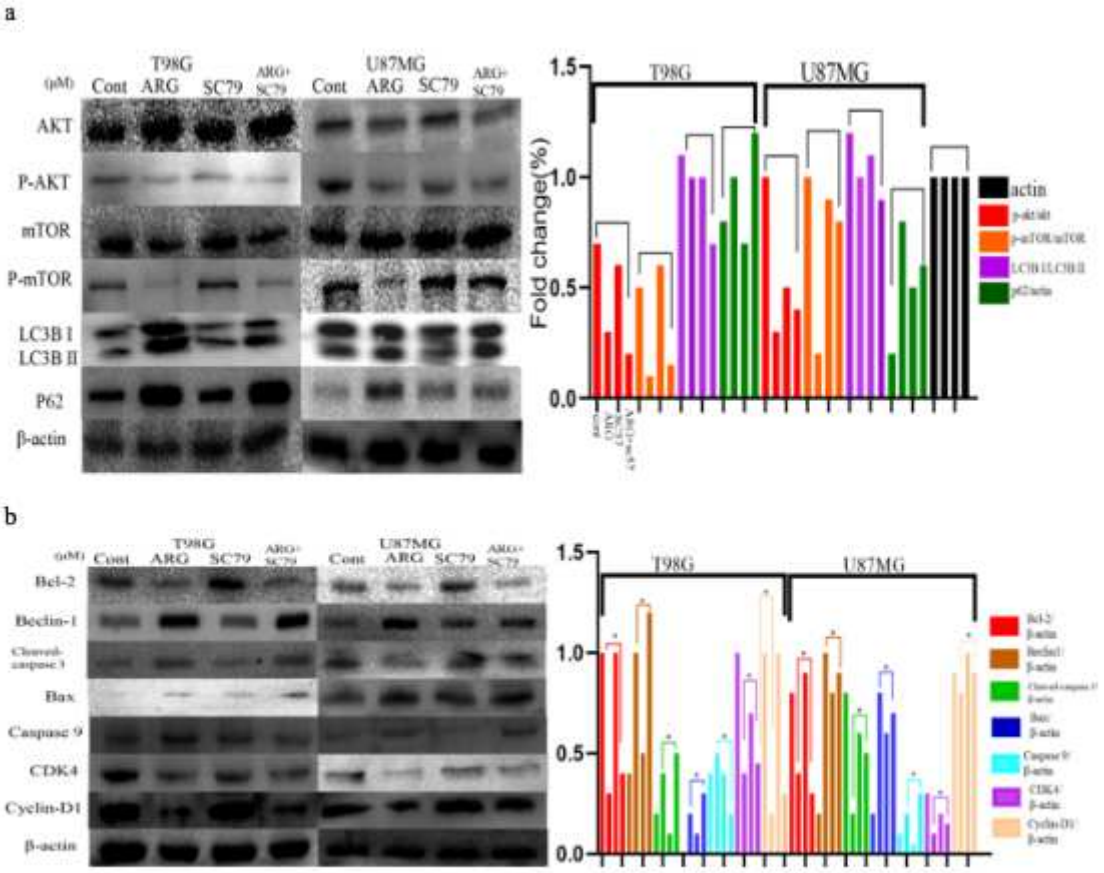
a
T98G
U87MG
(μM)
Cont
ARG
SC79
ARG+ SC79
AKT
P-AKT
mTOR
P-mTOR
LC3B I
LC3B II
P62
β-actin
Fold change(%)
1.5
1.0
0.5
0.0
actin
b
Bcl-2
Beclin-1
Bax
Caspase 9
CDK4
Cyclin-D1
β-actin

Figure 4