Experimental and Molecular Pathology 107 (2019) 95–101

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

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

Brazilein induces apoptosis and G1/G0 phase cell cycle arrest by up-regulation of miR-133a in human vestibular schwannoma cells

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ARTICLE INFO

Keywords:
Brazilein
Acoustic neuroma
miR-133a
G1/G0 cell cycle
MAPK and JNK pathways

ABSTRACT

Background: Acoustic neuroma is a benign and usually slow growing tumor. Brazilein is a natural compound extracted from hematoxylin. However, there has been no study of the mechanism of brazilein in acoustic neuroma cells. Thus, we aimed to investigate the effects and mechanism of brazilein on human VS cells in this study.
Methods: The vestibular schwannoma (VS) cells were collected from patient tissues and used in this study. Different concentrations of brazilein (0, 10, 20 and 30 μM) were used to treat VS cells. The expression of miR-133a was altered by transfection with miR-133a inhibitor. Further, cell viability, apoptosis, cell cycle, the mRNA and phosphorylation levels of cell cycle, apoptosis-related proteins and main factors in MAPK and JNK pathways were detected using CCK-8 assay, flow cytometry analysis, qRT-PCR and western blot analysis, respectively.
Results: The results showed that brazilein decreased cell viability, increased apoptosis and induced G1/G0 cell cycle arrest in VS Cells. Further, miR-133a expression was up-regulated in the brazilein-treated cells. Brazilein promoted apoptosis and induced G1/G0 cell cycle arrest via up-regulation of miR-133a. In addition, brazilein inhibited the activations of MAPK and JNK pathways by up-regulating miR-133a expression.
Conclusion: In conclusion, our study demonstrated that brazilein could induce apoptosis and G1/G0 phase cell cycle arrest, and deactivate MAPK and JNK signaling pathways via up-regulation of miR-133a in human VS cells. These results provide theoretical evidence for the clinical application of brazilein and a new strategy for the treatment of acoustic neuroma.

1. Introduction

Acoustic neuroma is also known as vestibular schwannoma (VS) and an uncommon cause of hearing loss (Jr et al., 1996). It occurs in the eighth cranialnerve sheath, which is mainly from the vestibular branch of the eighth cranialnerve (Webster et al., 2014). However, acoustic neuroma is a benign and usually slow growing tumor (Board, 2012), but in a few cases, it may increase rapidly and then oppress the brain and interfere with its important function (Xing et al., 2015). The most common early symptoms of acoustic neuroma are unilateral progressive sensorineural hearing loss and tinnitus (Lee et al., 2015), and later there will be facial numbness, facial paralysis and cranial hypertension (Stangerup et al., 2010). In recent years, the incidence of acoustic neuroma has shown an increasing trend (Stangerup et al., 2010). Then, the treatment methods of acoustic neuroma include routine monitoring, radiotherapy and surgical resection (Jalali, 2004). Moreover, drug-targeted therapy can not only control the growth of acoustic neuroma, but also avoid the damage of blood vessels and nerves caused by traditional surgery (De et al., 2015; Mautner et al., 2010). Traditional Chinese medicine treatments of acoustic neuroma have also been widely used in China (Cheng et al., 2016). Therefore, it is of great value to find a highly effective and safe Chinese herbal medicine for the treatment of acoustic neuroma.

Hematoxylin is a commonly used traditional Chinese medicine with the functions of activating blood, removing blood stasis, detumescence and relieving pain (Ke-Bo and Sun, 2013). Brazilein ($C_{16}H_{16}O_6$, Fig. 1A) is a natural compound extracted from hematoxylin (Tao et al., 2013). It is often used in the preparation of drugs for the treatment of cerebral ischemia or cerebral hemorrhage, brain tissue peroxidation and brain function changes (Hung et al.). A large numbers of researches reported that brazilein has a lot of pharmacological activity, such as anti-heart failure, immunosuppression, neuroprotection and anti-tumor, and then acts on a variety of diseases (Cao et al., 2011; Yen et al., 2010a; Yen et al., 2010b). For example, brazilein could inhibit the growth of breast cancer cells by regulating GSK-3b signaling pathway (Tao et al., 2013), protect the brain against focal cerebral ischemia reperfusion injury

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https://doi.org/10.1016/j.yexmp.2018.12.010
Received 29 August 2018; Received in revised form 29 November 2018; Accepted 31 December 2018
Available online 02 January 2019
0014-4800/

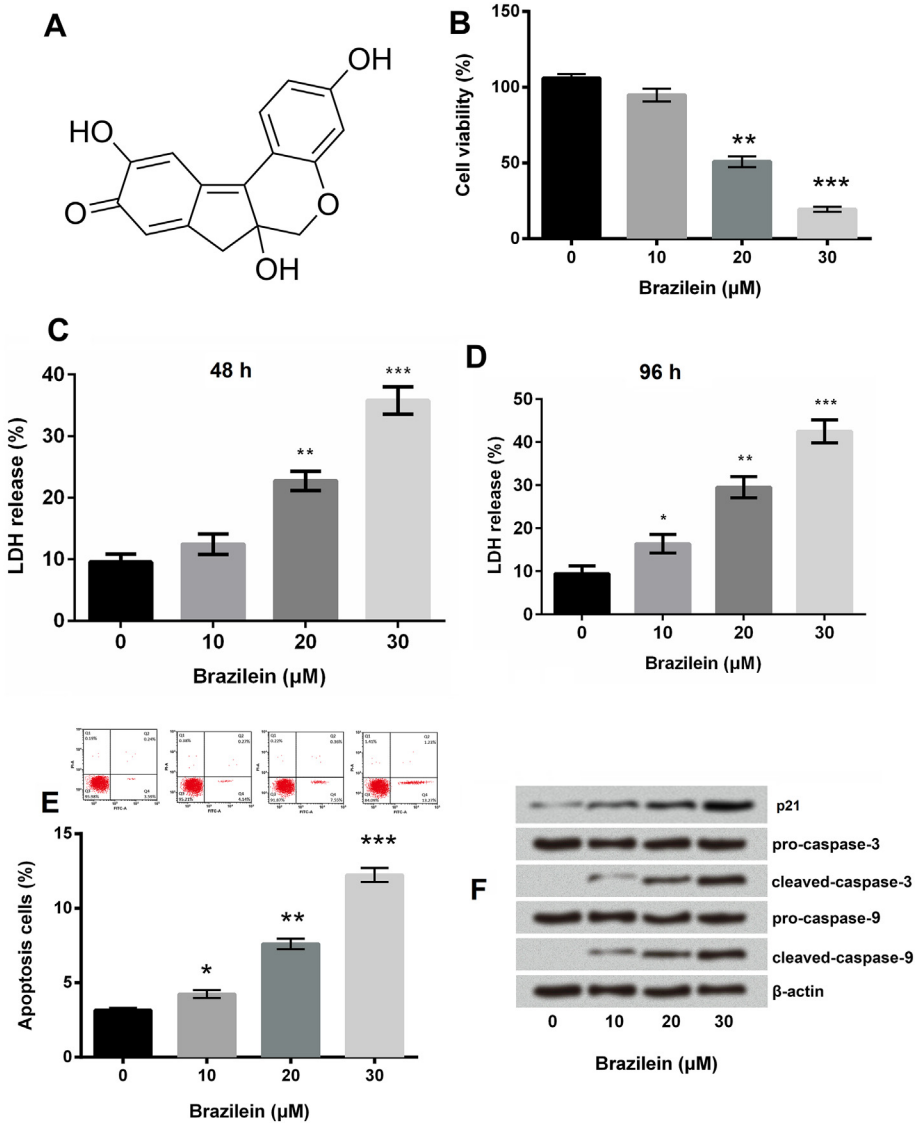

Fig. 1. Brazilein induced apoptosis in VS cells. (A) The chemical structural formula of brazilein. (B) Cell viability, (C) cell cytotoxicity at 48 h, (D) Cell cytotoxicity at 96 h(E) Apoptosis and (F) The phosphorylation levels of apoptosis-related proteins were detected at the different concentrations of brazilein (0, 10, 20 and 30 μM) by CCK-8 assay, LDH assay, flow cytometry analysis and western blot analysis, respectively. All data were presented by mean ± SD ($n = 3$). * $P < .05$; ** $P < .01$; *** $P < .001$.CCK-8, Cell Counting Kit-8. LDH, lactate dehydrogenase.

(Shen et al., 2007), and overcome ABCB1-mediated multidrug resistance in human leukaemia cells (Tao, 2011). However, the effects and regulation mechanisms of brazilein on the acoustic neuroma cells remain unclear.

Therefore, in this study, we aimed to explore the effects and molecular mechanism of brazilein on human VS cells to reveal the potentials of brazilein in acoustic neuroma therapy by focusing on cell viability, apoptosis, cell cycle and MAPK and JNK signaling pathways.

2. Materials and methods

2.1. Cells collection and culture

VS cells were collected from patients undergoing microsurgery in Yantai Hospital of Traditional Chinese Medicine between March 2015 to May 2016. Freshly harvest human specimens of VSs were washed three times with saline solution and then snap frozen in liquid nitrogen until use. This study was approved by the ethics committee of our local institution. Informed consent was obtained from each participant. Primary human VS cultures were prepared as previously described. In brief, VSs were cut into 1-mm^3 fragments, digested with 0.25% trypsin and 0.1% collagenases for 30–40 min at 37 °C and dissociated by trituration through fire-polished Pasteur pipettes. Following centrifugation at 300 × *g* for 5 min, the supernatant was discarded. The cells were resuspended in culture plates and cultured in DMEM containing 10% fetal bovine serum and N_2 additives. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Primary human VS cultures were not passaged prior to experimental manipulation. In addition, cells were treated with brazilein (Herbpurify Co., Chengdu, China) at different concentrations (0, 10, 20 and 30 μM) diluted with H_2O for 48 h.

2.2. Cell transfection

In order to detect the expression of miR-133a, miR-133a inhibitor and its negative control (NC) were synthesized by using GenePharma Co. (Shanghai, China). Then, they were transfected into cells by using lipofectamine 3000 reagent (Life Technologies Corporation, Carlsbad, USA) according to instruction of manufacturer. After 48 h transfection, the stably transfected cells were cultured for about 4 weeks on the

culture medium containing 0.5 mg/mL G418 (Sigma-Aldrich, St Louis, USA). G418-resistant cells were collected directly for subsequent experiments.

2.3. Cell viability

Collected stable cells were used to assess cell viability by using Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Gaithersburg, MD). Briefly, cells were seeded in 96-well plate with 5000 cells/well. After 48 h of brazilein stimulation, removed culture medium and cells were washed twice by PBS. Then the CCK-8 solution was added to the culture medium. Then, the cells were incubated for 1 h at 37 °C in humidified 95% air and 5% CO_2. The absorbance was measured at 450 nm by using a Microplate Reader (Bio-Rad, Hercules, USA).

2.4. Analysis of cytotoxicity

The cells were plated into a 96-well microplate at a density of 2×10^4 cells/well in the growth medium supplemented with 2% serum on the day before the experiment was performed. Following treatment with agents for 48 h and 96 h, the activity of lactate dehydrogenase (LDH) in medium was measured using a Cytotoxicity Detection Kit (Roche Diagnostics Ltd., Burgess Hill, UK) according to the manufacturer's instructions.

2.5. Apoptosis assay

Further, flow cytometry analysis was used to detect apoptosis by propidium iodide (PI) and fluorescein isothiocynate (FITC)-conjugated Annexin V staining. Firstly, cells treated by brazilein were washed in phosphatebuffered saline (PBS) twice and fixed in 70% ethanol. Then, fixed cells were washed PBS twice again, stained in PI/FITC-Annexin V in the presence of 50 μg/mL RNase A (all from Sigma-Aldrich, St Louis, USA), and incubated for 1 h at room temperature in the dark. Finally, FACS can (Beckman Coulter, Fullerton, USA) and was FlowJo software (TreeStar, Ashland, USA) were used to analyze the data.

2.6. Cell cycle assay

For analysis of cell cycle, cells were trypsinized, washed twice in PBS, and fixed in 100% ethanol overnight at −20 °C. The fixed cells were spun down gently in 200 μL Cell Extraction Buffer (Thermo Fisher Scientific, Waltham, USA) at 37 °C for 30 min, and then stained with 50 μg/mL of PI (Sigma-Aldrich, St Louis, USA) containing 50 μg/mL RNase A for 30 min at 37 °C in the dark, and subsequently analyzed by FACS can (Beckman Coulter, Fullerton, USA). The data were analyzed using Cell Questk and ModFitk software (Becton Dickinson, San Jose, CA, USA).

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA was extracted from cells and tissues by using RNA pure Rapid Extraction Kit (Bioteke Corporation, Beijing, China) following the manufacturer's instructions. The Taqman MicroRNA Reverse Transcription Kit and Taqman Universal Master Mix II with the TaqMan MicroRNA Assay of miR-133a and U6 (Applied Biosystems, Foster City, USA) were used for testing the expression levels of miR-133a in VS cells. Relative quantification analysis was conducted using the $2^{-\Delta\Delta Ct}$ method (Arocho et al., 2006).

2.8. Western blot assay

RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Basel, Switzerland) were used for extracting proteins, which were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, USA). The western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions. The antibodies of p21 (ab109520) pro-caspase-3 (ab32150), cleaved-caspase-3 (ab49822), pro-caspase-9 (ab135544), cleaved-caspase-9 (ab2324), CyclinD1 (ab137875), Cdk4 (ab68266) and β-action (ab8227) were purchased from Abcam (Shanghai, China). While the antibodies of t-MAPK (#9102), p-MAPK (#4370), t-JNK (#9252), p-JNK (#4668), t-c-Jun (#9165) and p-c-Jun (#3270) were obtained from Cell Signaling Technology (Danvers, USA). They were prepared in 5% blocking buffer at a dilution of 1:1000, and then incubated with the membrane at 4 °C overnight, followed by wash and incubation with secondary antibody marked by horseradish peroxidase for 1 h at room temperature. After rinsing, the Polyvinylidene Difluoride (PVDF) membrane carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and then 200 μL Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA, USA) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Shanghai, China).

2.9. Statistical analysis

All experiments were at least repeated three times. The results of multiple experiments were presented as the mean ± standard deviation (SD). Statistical analyses were performed by using Graphpad 6.0 software (GraphPad Software Inc., La Jolla, USA). The *P*-values were calculated using a one-way analysis of variance (ANOVA). *P*-value < .05 was considered to indicate a statistically significant result.

3. Results

3.1. Brazilein induced apoptosis in VS cells

In this study, we explored the effects of brazilein at different concentrations (0, 10, 20 and 30 μM) on VS cells by using CCK-8 assay, flow cytometry analysis and western blot analysis. The results of CCK-8 assay (Fig. 1B) showed that cell viability was significantly decreased in the 20 and 30 μM groups ($P < .01$ or $P < .001$). Then we detected the cell cytotoxicity under different concentrations of Brazilein and found that LDH release was significantly increased (20 μM, $P < .01$, 30 μM $P < .001$, Fig. 1C) at the time interval 48 h and similar results was observed at the time interval 96 h (10 μM, $P < .05$, 20 μM, $P < .01$ and 30 μM, $P < .001$, Fig. 1D).

On the other side, apoptotic cells were induced in the 10, 20 and 30 μM groups ($P < .05$, $P < .01$ or $P < .001$), especially the 30 μM group increased the most (Fig. 1E). Moreover, western blot analytical results (Fig. 1F) suggested that the phosphorylation levels of cleaved-caspase-3/9 and p21 were gradually up-regulated with elevated concentrations of brazilein. Overall, these findings revealed that brazilein could decrease cell viability and increase apoptosis in VS cells.

3.2. Brazilein induced G1/G0 phase cell cycle arrest in VS Cells

Next, we examined the effect and regulation of brazilein at different concentrations (0, 20 and 30 μM) on the cell cycle G1/G0 phase by using flow cytometry analysis, qRT-PCR and western blot analysis. From Fig. 2A, we found that 20 and 30 μM of brazilein increased cell number of G1/G0 phase compared to 0 μM of brazilein ($P < .05$ or $P < .01$). However, cell numbers of S phase and G2/M phase were reduced in the 20 and 30 μM group ($P < .05$ or $P < .01$). As shown in Fig. 2B and C, the mRNA and phosphorylation levels of cell cycle related proteins CyclinD1 and Cdk4 were obviously down-regulated with increased concentrations of brazilein ($P < .05$ or $P < .01$). Sum up, these results suggested that brazilein could induce G1/G0 phase cell cycle arrest in VS Cells.

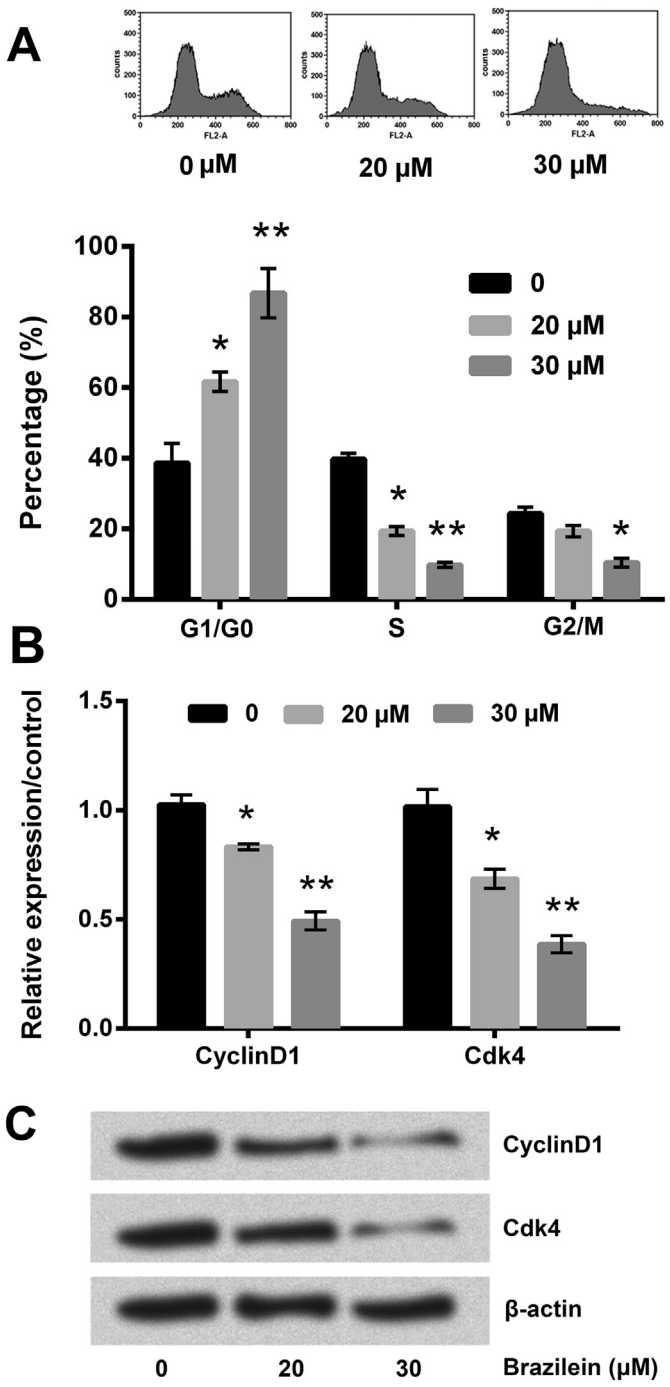

Fig. 2. Brazilein induced G1/S cell cycle arrest in VS Cells. (A) The cell number percentage of G1/G0, S and G2/M phase, (B–C) The expressions of cell cycle related proteins were measured at the different concentrations of brazilein (0, 20 and 30 μM) by using flow cytometry analysis, qRT-PCR and western blot analysis, respectively. All data were presented by mean ± SD (n = 3). * $P < .05$; ** $P < .01$.

3.3. Brazilein promoted apoptosis and induced G1/G0 cell cycle arrest via up-regulation of miR-133a

Then, the expression of miR-133a was analyzed in VS cells treated with different concentrations (0, 10, 20 and 30 μM) of brazilein by using qRT-PCR. The results (Fig. 3) displayed that miR-133a was overexpressed when brazilein concentrations were 20 and 30 μM ($P < .05$ or $P < .01$). Further, 30 μM of brazilein was selected to use in the subsequent experiments.

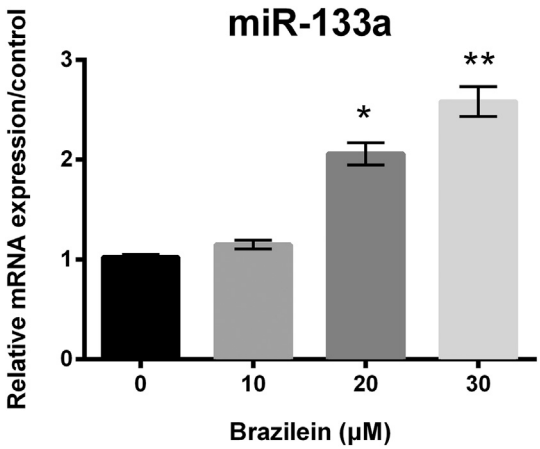

Fig. 3. The miR-133a expression was up-regulated in brazilein-treated cells. The mRNA level of miR-133a at the different concentrations of brazilein (0, 10, 20 and 30 μM) was analyzed by using qRT-PCR. All data were presented by mean ± SD (n = 3). * $P < .05$; ** $P < .01$.

Subsequently, in order to detect the effects of miR-133a on brazilein, cells were transfected with miR-133a inhibitor. From Fig. 4A, we found that the transfection of miR-133a inhibitor was successful ($P < .01$). Then, flow cytometry analytical results (Fig. 4B) showed that apoptosis was decreased in VS cells treated by 30 μM brazilein plus miR-133a suppression ($P < .05$). Similarly, the apoptosis-related proteins cleaved-caspase-3/9 expressions and p21 were significantly down-regulated in the brazilein + miR-133a inhibitor group (Fig. 4C). Furthermore, the increase cell number of G1/G0 phase and reduction of S and G2/M phase were abolished by miR-133a suppression ($P < .05$, Fig. 4D). As shown in Fig. 4E and E, CyclinD1 and Cdk4 expressions were also clearly up-regulated in the brazilein + miR-133a inhibitor group ($P < .01$). From the above results, brazilein could promote apoptosis and induced G1/G0 cell cycle arrest via up-regulation of miR-133a.

3.4. Brazilein inhibited the activations of MAPK and JNK pathways via up-regulating miR-133a expression

In addition, the effects of MAPK and JNK pathways on brazilein were analyzed using qRT-PCT and western blot assay. The results in Fig. 5A-5D, the expressions of p-MAPK, p-JNK and p-c-Jun were all down-regulated after brazilein treatment ($P < .05$ or $P < .01$). However, miR-133a suppression inhibited these down-regulations ($P < .05$ or $P < .01$). These results suggested that brazilein could inhibit the activations of MAPK and JNK pathways via up-regulating miR-133a expression.

4. Discussion

Acoustic neuroma is one of the common intracranial tumors. Then, brazilein is a compound extracted from hematoxylin. In this study, we preliminary investigated the effects and regulation mechanism of brazilein on human VS cells. We found that brazilein decreased cell viability, increased apoptosis and induced G1/G0 cell cycle arrest in VS Cells. Then, brazilein promoted miR-133a overexpression in the VS cells. Furthermore, the results showed that brazilein promoted apoptosis and induced G1/G0 cell cycle arrest via up-regulation of miR-133a. In addition, brazilein inhibited the activations of MAPK and JNK signaling pathways through up-regulating miR-133a expression.

Previous studies reported that brazilein suppressed cell migration and invasion, induced apoptosis in many kinds of cancers, such as breast cancer and hepatocellular carcinoma (Hsieh et al., 2013; Zhong et al., 2009). The results of our research were consistent with previous

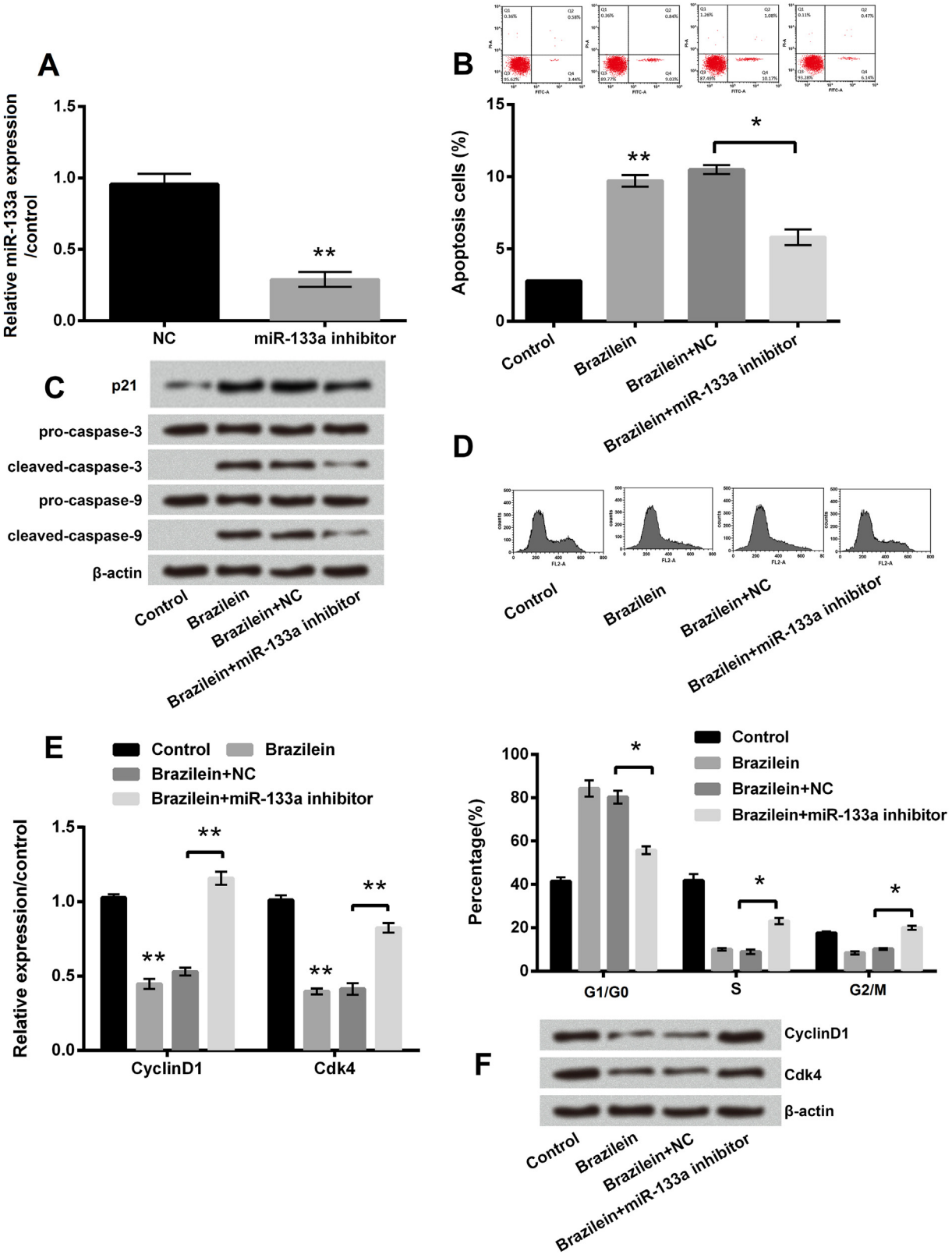

Fig. 4. Brazilein promoted apoptosis and induced G1/G0 cell cycle arrest via up-regulation of miR-133a. Cells were transfected with miR-133a inhibitor and treated with 30 μM of brazilein. (A) The mRNA level of miR-133a was analyzed by using qRT-PCR. (B) Apoptosis and (C) The phosphorylation levels of apoptosis-related proteins were detected by flow cytometry analysis and western blot analysis, respectively. (D) The cell number percentage of G1/G0, S and G2/M phase were measured by flow cytometry analysis. (*E*-F) The expressions of cell cycle related proteins were analyzed by using qRT-PCR and western blot analysis, respectively. All data were presented by mean ± SD (n = 3). * $P < .05$; ** $P < .01$.

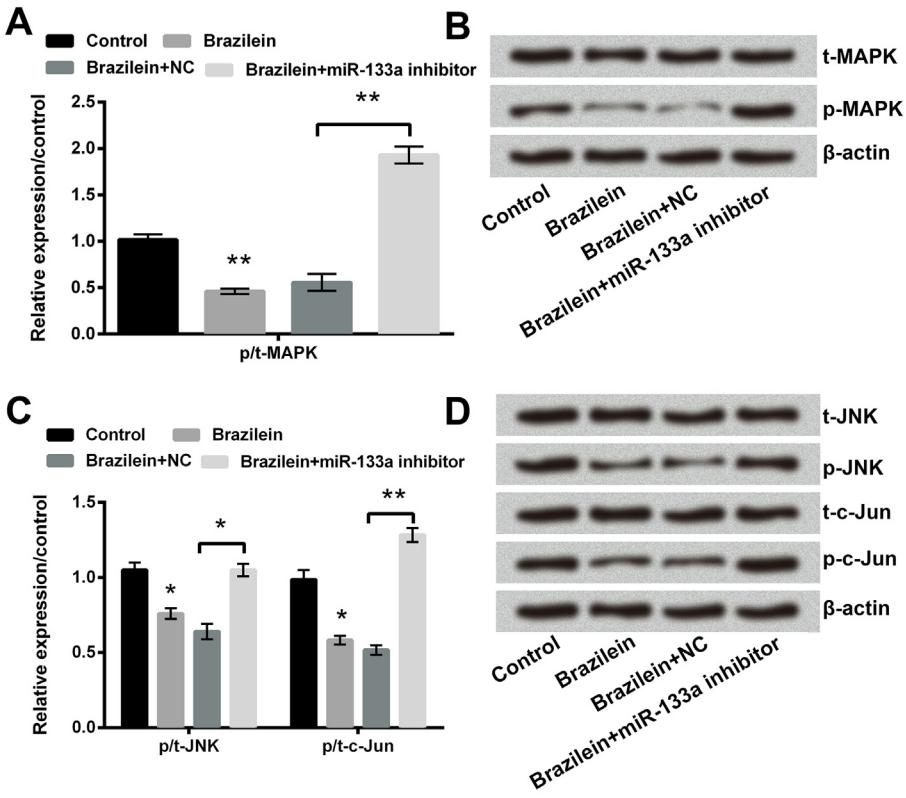

Fig. 5. Brazilein inhibited the activations of MAPK and JNK pathways via up-regulating miR-133a expression. Cells were transfected with miR-133a inhibitor and treated with 30 μM of brazilein. The mRNA and phosphorylation levels of main factors in (A-B) MAPK and (C-D) JNK signaling pathways were measured by qRT-PCR and western blot analysis, respectively. All data were presented by mean ± SD (n = 3). * $P < .05$; ** $P < .01$.

studies, that is, brazilein decreased cell viability and increased apoptosis in VS cells. Furthermore, Tao et al suggested brazilein was involved in CyclinD1 pathway to inhibit cell growth (Tao et al., 2013), thus we detected the effects of brazilein on cell cycle in VS cells. The regulatory mechanism of cell cycle plays an important role in pathogenesis, clinical diagnosis and treatment of cancers (Kamb et al., 1994). The most important regulators are Cyclin and cyclin-dependent kinases (CDKs), which are the core of the regulatory network (Kamb et al., 1994). We chose CyclinD1 and Cdk4 to analyze the influence of brazilein in different phases and found that brazilein could induce G1/G0 cell cycle arrest in VS cells.

On the other side, p21, which is CDK inhibitor, plays important roles in promoting cell cycle arrest in response to a variety of stimuli (Gongpan et al., 2016). Recent years, the functions of p21 in apoptosis received considerable attention (Karimian et al., 2016). Study from Chen et al. reported that p21 overexpression suppresses apoptosis in UVB-irradiated HaCaT keratinocytes but promotes apoptosis in non-irradiated HaCaT keratinocyte (Chen et al., 2015). The functions of p21 in apoptosis still need further explored. Study from Kim et al. found that p53/p21 complex can regulate cancer cell apoptosis (Kim et al., 2017). However, other report demonstrated that involvement of p21 independently of p53 (Pagliara et al., 2016; Russo et al., 2016). In our study, we found that Brazilein induced the expression of p21, which get along with increasing apoptosis. These results demonstrated that p21 overexpression promoted VS cell apoptosis. Cleaved-Caspase-3 and cleaved-Caspase-9 were two kinds of pro-apoptotic proteins (Hu et al., 2014; Wang et al., 2017). In our study, we found that brazilein promoted cleaved-Caspase-3 and cleaved-Caspase-9 expression, which consistent with the results that brazilein promoted apoptosis.

MicroRNAs (miRNAs) are non-coding RNAs with a length of about 22 nt and can participate in the proliferation, apoptosis, differentiation and cell cycle (Garzon et al., 2009). Dysfunction of the miRNA signals are present in all cancers studied so far that leads to oncogenic or non-active tumor suppressor gene signaling (Wilmott et al., 2011). Some studies found that miR-133a inhibited cell proliferation, migration, invasion and cell cycle, and had a tumor suppressive function in many cancers, such as gastric cancer, head and neck squamous cell carcinoma and head and neck squamous cell carcinoma (Gao et al., 2016; Hanazawa et al., 2010; Qiu et al., 2014). In our study, we found that miR-133a was overexpression in brazilein-treated VS cells. Thus, we hypothesized that miR-133a was involved in the regulation of acoustic neuroma by brazilein. Then, further study found that brazilein promoted apoptosis and induced G1/G0 cell cycle arrest via up-regulation of miR-133a.

Nowadays, Ras-related C3 botulinum toxin substrate (Rac)/c-Jun N-terminal kinase (JNK) and its downstream pathway are hotspots of various schwannomas. Some researchers speculated that the lack of Merlin protein could lead to the activation of Rac, which causes downstream Rac-dependent mitogen-activated protein kinase (MAPK) signaling pathway activation and increases the phosphorylation level of JNK (Hexter and Evans, 2014). Kaempchen et al (Kaempchen et al., 2003) found that Rac activation was significantly up-regulated and the phosphorylation level of JNK was enhanced by comparing normal blood cells with schwannoma cells. These results suggested that activation of Rac/JNK and MAPK signaling pathways may be involved in the pathogenesis of schwannoma.

Therefore, we were mainly concerned about the effects of JNK and MAPK signaling pathways on brazilein-treated cells. Our results exhibited that the main factors p-MAPK, p-JNK and p-c-Jun were down-regulated after brazilein conditioned. But these down-regulations were abolished when miR-133a knockdown. Such results hinted that brazilein deactivated MAPK and JNK pathways via up-regulating miR-133a expression.

5. Conclusion

In conclusion, our study demonstrated that brazilein could induce apoptosis and G1/G0 phase cell cycle arrest, and deactivate MAPK and JNK signaling pathways through up-regulation of miR-133a in human VS cells. Data from this study directly linked brazilein to VS cells,

however, effects of brazilein should be strengthened by additional experiments in normal cell lines. These results might provide theoretical evidence for the clinical application of brazilein and a new strategy for the treatment of acoustic neuroma.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Arocho, A., et al., 2006. Validation of the 2-DeltaDeltaCt calculation as an alternate method of data analysis for quantitative PCR of BCR-ABL P210 transcripts. Diagn. Mol. Pathol. 15, 56–61.

Board, A. D. A. M. E., 2012. (Acoustic neuroma. A.d.a.m).

Cao, J., et al., 2011. Immunosuppression of NF-κB by intragastric brazilein in motor neuron of spinal cord connected with injured sciatic nerve in mouse. Biomed. Res. 23, 199–206.

Chen, A., et al., 2015. The Role of p21 in Apoptosis, Proliferation, Cell Cycle arrest, and Antioxidant activity in UVB-Irradiated Human HaCaT Keratinocytes. Med Sci Monit Basic Res. 21, 86–95.

Cheng, S.U., et al., 2016. Characteristics of Left and right Acoustic Neuroma in male and Female patients:A Comparative Analysis of 136 cases based on Traditional Chinese Medicine and Western Medicine. Journal of Anhui University of Chinese Medicine 36, 36–39.

De, V.M., et al., 2015. Tumor Biology of Vestibular Schwannoma: a Review of Experimental Data on the Determinants of Tumor Genesis and Growth Characteristics. Otology & neurotology 36, 1128.

Gao, S.H., et al., 2016. Low miR-133a expression is a predictor of outcome in patients with esophageal squamous cell cancer. Eur. Rev. Med. Pharmacol. Sci. 20, 3788.

Garzon, R., et al., 2009. MicroRNAs in Cancer. Annu. Rev. Med. 60, 167–179.

Gongpan, P., et al., 2016. AS160 controls eukaryotic cell cycle and proliferation by regulating the CDK inhibitor p21. Cell Cycle 15, 1733–1741.

Hanazawa, T., et al., 2010. Elucidation of tumor suppressive function of miR-145, miR-133a and miR-133b and identification of their target genes in head and neck squamous cell carcinoma. Japanese Journal of Head & Neck Cancer. 36, 1–8.

Hexter, A.T., Evans, D.G., 2014. The Genetics of Vestibular Schwannoma. Current Otorhinolaryngology Reports. 2, 226–234.

Hsieh, C.Y., et al., 2013. Brazilein suppresses migration and invasion of MDA-MB-231 breast cancer cells. Chem. Biol. Interact. 204, 105–115.

Hu, Q., et al., 2014. Elevated cleaved caspase-3 is associated with shortened overall survival in several cancer types. Int. J. Clin. Exp. Pathol. 7, 5057–5070.

Jalali, R., 2004. High Precision Radiotherapy Techniques in the Management of Brain Tumours: Evolution and Clinical experience. Jk Science Journal of Medical Education & Research. 6.

Jr, N.J., et al., 1996. Correlation of hearing loss and radiologic dimensions of vestibular schwannomas (acoustic Neuromas). Am. J. Otolaryngol. 17, 312.

Kaempchen, K., et al., 2003. Upregulation of the Rac1/JNK signaling pathway in primary human schwannoma cells. Hum. Mol. Genet. 12, 1211–1221.

Kamb, A., et al., 1994. A cell cycle regulator potentially involved in genesis of many tumor types. Science 264, 436–440.

Karimian, A., et al., 2016. Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. DNA Repair (Amst) 42, 63–71.

Ke-Bo, G.U., Sun, G.Z., 2013. Effect of hematoxylin and its extract on immune system. China Journal of Traditional Chinese Medicine & Pharmacy 28, 2360–2362.

Kim, E.M., et al., 2017. The p53/p21 complex Regulates Cancer Cell Invasion and Apoptosis by Targeting Bcl-2 Family Proteins. Cancer Res. 77, 3092–3100.

Lee, S.H., et al., 2015. Otologic manifestations of acoustic neuroma. Acta Otolaryngol. 135, 140.

Mautner, V.F., et al., 2010. Bevacizumab induces regression of vestibular schwannomas in patients with neurofibromatosis type 2. Neuro-Oncology 12, 14.

Pagliara, V., et al., 2016. 5-FU targets rpL3 to induce mitochondrial apoptosis via cystathionine-beta-synthase in colon cancer cells lacking p53. Oncotarget 7, 50333–50348.

Qiu, T., et al., 2014. MiR-145, miR-133a and miR-133b inhibit proliferation, migration, invasion and cell cycle progression via targeting transcription factor Sp1 in gastric cancer. FEBS Lett. 588, 1168.

Russo, A., et al., 2016. rpL3 promotes the apoptosis of p53 mutated lung cancer cells by down-regulating CBS and NFkappaB upon 5-FU treatment. Sci. Rep. 6, 38369.

Shen, J., et al., 2007. Brazilein protects the brain against focal cerebral ischemia reperfusion injury correlating to inflammatory response suppression. Eur. J. Pharmacol. 558, 88.

Stangerup, S.E., et al., 2010. True Incidence of Vestibular Schwannoma? Neurosurgery. Vol. 67. pp. 1335.

Tao, L., 2011. Brazilein overcame ABCB1-mediated multidrug resistance in human leukaemia K562/AO2 cells. Afr. J. Pharm. Pharmacol 5, 1937–1944.

Tao, L.Y., et al., 2013. Brazilein, a compound isolated from Caesalpinia sappan Linn., induced growth inhibition in breast cancer cells via involvement of GSK-3beta/beta-Catenin/cyclin D1 pathway. Chem. Biol. Interact. 206, 1–5.

Wang, F., et al., 2017. Apoptosis Inducing factor is involved in Stretch-Induced Apoptosis of Myoblast via a Caspase-9 Independent Pathway. J. Cell. Biochem. 118, 829–838.

Webster, G., et al., 2014. International Archives of Otorhinolaryngology. International Archives of Otorhinolaryngology. 337.

Wilmott, J.S., et al., 2011. The emerging important role of microRNAs in the pathogenesis, diagnosis and treatment of human cancers. Pathology 43, 657.

Xing, H.S., 2015. Protection of facial nerves during acoustic neuroma surgery. Cell Biochem. Biophys. 72 (1), 73–76.

Yen, C.T., et al., 2010a. ChemInform Abstract: Antitumor Agents. Part 271. Total Synthesis and Evaluation of Brazilein and Analogues as Antiinflammatory and Cytotoxic Agents. Cheminform. 41. In: no-no.

Yen, C.T., et al., 2010b. Antitumor Agents. 271. Total Synthesis and Evaluation of Brazilein and Analogs as Anti-inflammatory and Cytotoxic Agents. Bioorg. Med. Chem. Lett. 20, 1037–1039.

Zhong, X., et al., 2009. Brazilein inhibits survivin protein and mRNA expression and induces apoptosis in hepatocellular carcinoma HepG2 cells. Neoplasma 56, 387–392.