Silibinin Promotes Cell Proliferation Through Facilitating G1/S Transitions by Activating Drp1-Mediated Mitochondrial Fission in Cells

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Abstract
Heart, liver, and kidney, which are known as the essential organs for metabolism, possess the unique ability to regulate the proliferation function of the body against injury. Silibinin (SB), a natural polyphenolic flavonoid extracted from traditional herb *Silybum marianum* L., has been used to protect hepatocytes. Whether SB can regulate mitochondrial fission in normal cells and the underlying mechanisms remain unclear. Here, we showed that SB markedly promoted cell proliferation by facilitating G1/S transition via activating dynamin-related protein 1 (Drp1), which in turn mediated mitochondrial fission in these normal cells. SB dose-dependently increased the mitochondrial mass, mtDNA copy number, cellular adenosine triphosphate production, mitochondrial membrane potential, and reactive oxygen species in normal cells. Furthermore, SB dose-dependently increased the expression of Drp1. Blocking Drp1 abolished SB-induced mitochondrial fission. In conclusion, we demonstrate that SB promotes cell proliferation through facilitating G1/S transition by activating Drp1-mediated mitochondrial fission. This study suggests that SB is a potentially useful herbal derivative for the daily prevention of various diseases caused by impaired mitochondrial fission.

Keywords
silibinin, G1/S cell cycle, mitochondrial fission, dynamin-related protein 1

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Introduction

*Silybum marianum* L. has been used to treat liver diseases for centuries. Silibinin (SB), a major component of flavonolignans mixture in *Silybum marianum* L., has been identified with antioxidant, hepatoprotective, neuroprotective, cardioprotective, avoid hepatitis C virus (HCV) reinfection, and anticancer effects.

Mitochondria are essential eukaryotic organelles that provide energy for the majority of processes including metabolism, cell cycle progression, differentiation, immune responses, and apoptotic cell death. Under physiological conditions, the mitochondrial network emerges highly dynamic modulating bioenergetics, such as reactive oxygen species (ROS) generation, cell proliferation, and death. Dysfunction in mitochondrial dynamics results in impaired adenosine triphosphate (ATP) synthesis, decreased mitochondrial membrane potential (MMP), mitochondrial DNA (mtDNA) mutation, and excessive ROS production, which causes various diseases, including cardiovascular diseases, kidney diseases, metabolic diseases, and cancer. Mitochondrial fission is essential for maintaining the mitochondrial network. Dynamin-related protein 1 (Drp1), a large dynamic-related cytosolic GTPase, is recruited to mitochondrial outer membrane and forms as active GTP-dependent mitochondrial fission sites during fission. It has been reported that dysfunctional Drp1 can disrupt mitochondrial homeostasis and lead to cell death.

The restoration of Drp1-mediated mitochondrial fission might be a mechanism underlying SB protecting against cardiac, hepatic, or nephritic diseases. This hypothesis has not been fully validated. In this study, we used cardiomyocyte, hepatocyte, and renal tubular epithelial cell models to demonstrate that SB can increase mitochondrial form and function by restoring Drp1-mediated mitochondrial fission.

Materials and Methods

Cell Line and Culture

The human AC16 cardiomyocytes (Cellcook Biotech Co., Ltd., Guangzhou, China) were cultured in Dulbecco’s modified Eagle medium (high glucose, GIBCO BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (BI, Beit Haemek, Israel), penicillin (100 U/ml, BI), and streptomycin (100 µg/ml, BI). The human LO2 hepatocytes (Cellcook Biotech Co., Ltd.) were cultured in RPMI-1640 (GIBCO BRL), supplemented with 10% fetal bovine serum (BI), penicillin (100 U/ml, BI), and streptomycin (100 µg/ml, BI). And the human proximal tubular epithelial HK2 cell line was cultured in Dulbecco’s modified Eagle medium/F12 (GIBCO BRL), supplemented with 10% fetal bovine serum (BI), penicillin (100 U/ml, BI), and streptomycin (100 µg/ml, BI). All cells were maintained at 37°C and 5% CO₂ in a humid environment. Cells in the mid-log phase were used in subsequent experiments.

Cell Viability and Cell Growth Assay

The effects of SB (Chengdu Must Bio-Technology Co., Ltd., Chengdu, China) on cell viability were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide (MTT). LO2 (3 x 10³ cells/well) cells, AC16 (3 x 10³ cells/well) cells, and HK2 (5 x 10³ cells/well) cells were seeded onto 96-well microplate and cultured for 24 h. The cellular viability was assessed using MTT assays and was expressed as a ratio to the absorbance value at 570 nm of the control cells by a microplate reader (Multiskan FC, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Colony Formation Assay

LO2 (500 cells/well) cells, AC16 (500 cells/well) cells, and HK2 (500 cells/well) cells were seeded onto six-well plates and cultured for 24 h then treated with SB at indicated concentrations for indicated periods (24, 48, and 72 h). The cellular viability was assessed using MTT assays and was expressed as a ratio to the absorbance value at 570 nm of the control cells by a microplate reader (Multiskan FC, Thermo Fisher Scientific, Inc., Waltham, MA, USA).
Cell Proliferation Assays

KeyFluor488 Click-iT EdU kit (KeyGen BioTECH, Nanjing, China) was used to detect SB-induced (0, 12.5, 25, and 50 μM/l) proliferation changes. Nuclear was stained with Hoechst 33342 (1 µg/ml) for cellular localization. After washing with PBS, samples were visualized at ×40 magnification (Olympus IX53, Tokyo, Japan).

Flow Cytometry of Cell Cycle

The cell cycle was measured by Cell Cycle Detection Kit (KeyGen BioTECH, Nanjing, China). Cells were harvested after 24 h of SB (0, 12.5, 25, and 50 μM/l) treatment, washed with PBS twice, and fixed with 70% ethanol at 4°C overnight. Cells were washed twice with PBS and incubated with RNase A for 30 min, then stained with Protiodium iodide (PI) in the darkroom. The cell cycle was analyzed by flow cytometry (CytoFLEX, Backman Counter, Bria, CA, USA).

Determination of Relative mtDNA Copy Number

The total DNA of SB treatment cells (0, 12.5, 25, and 50 μM/l) was extracted using TRIzol reagents and then subjected to qPCR analysis to determine the relative mtDNA copy number. The qPCR amplification reaction was performed via SYBR Green chemistry using LightCycler® 96 Real-time PCR system (Roche, Basel, Switzerland). The mtDNA was synthesized and amplified according to the manufacturer’s instructions as described previously.

Measurement of MMP

MMP was determined using MMP assay kit with JC-1 (Beayotime Institute of Biotechnology, Haimen, China) as described according to the manufacturer’s instruction. For each group (0, 12.5, 25, and 50 μM/l of SB treatment), JC-1 reagent was added and incubated for 20 min at 37°C. Cells were washed twice with PBS and detected by the fluorescence microscopy (Olympus FV1000, Tokyo, Japan). Living cells exhibited red fluorescent, whereas dead or dying cells exhibited green fluorescence.

Measurement of Intracellular ROS and ATP

The intracellular ROS levels of each group (0, 12.5, 25, and 50 μM/l of SB treatment) was determined using a ROS assay kit (Beyotime Institute of Biotechnology). Cells were stained with fluorescence dye DCFH-DA (10 μM/l) for 20 min in a darkroom and detected with flow cytometry. Cellular ATP levels were measured using an ATP Assay Kit (Beyotime Institute of Biotechnology). The assay is based on luciferase’s requirement (PerkinElmer, Waltham, MA, USA) for ATP in producing light. Luminescence was read, and values were calculated based on an ATP standard curve.

Immunofluorescent Staining

Cells of each group (0, 12.5, 25, and 50 μM/l of SB treatment) were fixed in 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 for 15 min, blocked with 5% bovine serum albumin in PBS for 1 h, and incubated with MitoRed (KeyGEN BioTECH, Jiangsu, China) for 1 h in darkness at room temperature (RT) to detect the mitochondrial morphology. Then, cells were incubated with 4',6-diamidino-2-phenylindole (Beayotime Institute of Biotechnology) in darkness at RT for 5 min. Samples were washed twice with PBS and imaged under a confocal microscope (LSM800, Carl Zeiss, Oberkochen, Germany).

RNA Extraction and qPCR

After 24 h treatment of SB (0, 12.5, 25, and 50 μM/l), cells were collected. Total cellular RNA was extracted from cells using TRIzol reagents and then subjected to qPCR analysis by SYBR® Premix Ex TaqTM II (Tli RNaseH Plus, TaKaRa, Tokyo, Japan). β-Actin was used as internal controls.

Western Blotting

The antibodies against Drp1, CDK2, cyclin E1, and β-actin were purchased from Affinity Biosciences (OH, USA). For western blotting, cells of each group (0, 12.5, 25, and 50 μM/l of SB treatment) were harvested and lysed with RIPA (Beyotime Institute of Biotechnology) buffer for 30 min, then centrifuged at 12,000×g for 15 min and the supernatant was collected. The proteins were quantified by the BCA Protein assay kit (Thermo Fisher Scientific, Inc.). The protein levels of Drp1, CDK2, cyclin E1, and β-actin from the cells were measured by the FluorChem E™ system (ProteinSimple, San Francisco, CA, USA).

Statistical Analysis

All data are expressed with the means ± standard deviations and analyzed by SPSS20.0 (IBM, Armonk, NY, USA). Multiple comparisons were analyzed by Tukey’s test. The values were considered statistically significant when \( P < 0.05 \).

Results

SB Promoted Cell Proliferation in Human AC16 Cardiomyocytes, LO2 Hepatocytes, and Human Proximal Tubular Epithelial HK2 Cells

Cell proliferation experiments were used to evaluate the potential effects of SB on normal cell progression. As shown in Fig. 1A, SB increased the viability of human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells in a dose- and time-dependent manner. We further detected the proliferation of normal cells by the colony formation assay and EdU incorporation assay after...
Fig. 1. SB promotes human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cell proliferation. (A) Cells were exposed to SB at different concentrations for 24, 48, and 72 h, and cell viability was measured by MTT assays. (B) SB promoted the proliferation of AC16, LO2, and HK2 cells. (C) Cell proliferation was detected by the keyFluor488 Click-iT EdU kit. Values (mean ± SDs) were obtained from at least three independent experiments. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide; SB: silibinin; SD: standard deviation.
SB treatment and found that SB significantly increased the normal cell proliferation (Fig. 1B, C). To further investigate the role of SB in the proliferation of AC16, LO2, and HK2 cells, we assayed the cell cycle by flow cytometry. The analysis of the cell cycle showed that the ratio of cells at the G1 to S phase was distinctly increased by SB (Fig. 2A). CDK2 and cyclinE1 protein levels were significantly increased in the normal cells treated with SB in dose-dependent manners (Fig. 2B). Taken together, these results suggest that SB could promote normal cell proliferation in vitro.

SB Increased Mitochondrial Function in Human AC16 Cardiomyocytes, LO2 Hepatocytes, and Human Proximal Tubular Epithelial HK2 Cells

Mitochondrial function plays a pivotal role in cell progression. Mitochondria are highly dynamic organelles that produce ATP to provide cellular energy. To investigate the effect of SB on mitochondrial form and function, the production of ATP was evaluated. Our results revealed that SB dose-dependently promoted ATP production in AC16, LO2, and HK2 cells (Fig. 3A). MMP, closely related to cellular ATP production, was significantly increased in AC16, LO2, and HK2 cells in a dose-dependent manner (Fig. 3B). Maintenance of the mtDNA copy number is essential for the preservation of mitochondrial form and function. The results showed that the mtDNA copy number was dramatically increased in a dose-dependent manner upon SB treatment (Fig. 3C). Mitochondria are the primary source of ROS in most cells. Moderate levels of ROS are needed to maintain the function of normal cells. These results showed that the level of ROS was increased in normal cells treated with SB (Fig. 3D). Hence, our findings suggest that SB could improve the mitochondrial function in AC16, LO2, and HK2 cells.

SB Promotes Cell Proliferation Through Drp1-Mediated Mitochondrial Fission in Human AC16 Cardiomyocytes, LO2 Hepatocytes, and Human Proximal Tubular Epithelial HK2 Cells

The highly mitochondrial dynamic network is tightly regulated by mitochondrial fission. MitoRed staining was used to observe the mitochondrial morphology. More fluorescence intensity and excessive mitochondrial fragmentation were observed in normal cells treated with SB (Fig. 4A). Then, we would like to find out whether Drp1 plays a role in SB-induced mitochondrial fission. As shown in Fig. 4B, SB treatment substantially increased the protein expression of Drp1 in a dose-dependent manner. The gene level of Drp1 was also increased in normal cells with the treatment of SB (Fig. 4C).

We then investigated the underlying mechanism of SB promoting cell proliferation by Drp1. Drp1-specific siRNA was used to knock down the expression of Drp1 in cells. As shown in Fig. 5A, Drp1-siRNA efficiently reduced the expression of Drp1 in cells. Drp1 has been proved as a major regulator in the cell cycle. Compared with the negative control group, SB significantly decreased the percentage of cells in G1/S phase (Fig. 6A), and reversed the increase of CDK2 and cyclinE1 protein expressions in Drp1-siRNA-transfected cells (Fig. 5B). Similarly, colony formation assay and EdU incorporation assay revealed that silencing Drp1 could reverse the pro-proliferative effects of SB in cells (Fig. 7A, B). These results suggest that SB promotes cell proliferation through up-regulating Drp1, which is an essential mediator in mitochondrial fission.

Discussion

As the essential metabolic organs of the body, heart, liver, and kidney have their unique capacity to regulate their growth and mass. However, it may lead to a cause of death when these metabolic organs get compromised and disable in the body. Thus, the potential for regeneration of these metabolic organs could promote a quick patch-up repair. SB, a predominant flavonoid component extracted from the fruits and seeds of *S. marianum* L, has proposed to have hepatoprotection, cardioprotection, metabolic syndrome alleviation, and anticancer effects. Recently, studies reported that SB can affect the function of mitochondria, but the mechanisms are still not clear. In this study, we are the first to demonstrate that SB could dose-dependently increase cell proliferation in human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells. The increased ATP content, mtDNA copy number, MMP, and ROS formation indicated that SB could benefit the mitochondrial formation and function. Moreover, we also found that SB promoted Drp1-mediated mitochondrial fission to improve mitochondrial function and formation. These data collectively suggest that SB treatment promotes human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells proliferation through Drp1-mediated mitochondrial fission.

In eukaryotic cells, the cell cycle plays a vital role in cell proliferation. During the G1 phase of the cell cycle, energy and biosynthetic capacity are accumulated for the duplication of the genome and cellular biomass. The G1/S transcriptional program initiates at the late G1 phase, in which DNA and protein synthesis are prepared for beginning a new round of proliferation. Cyclins and cyclin-dependent kinases (CDKs) are checkpoints to monitor the cell cycle progression. In mammalian cells, cyclinE1 is an activator of CDK2, and the active peak of cyclinE1/CDK2 kinase complex is required for cell cycle transition from the G1 phase to the S phase. In the present study, we found that SB promoted cell cycle G1/S transition and increased the protein expressions of CDK2 and cyclinE1 in human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells, which
Fig. 2. SB promotes G1 to S cell cycle transition in human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells. (A) A marked increase of the percentage of normal cells in the G2/M phase arrest by flow cytometry. Cells were stimulated in the presence or absence of SB at different doses for 24 h in six-well plates. (B) The protein expression levels of CDK2 and cyclin E1 at 24 h after SB treatment. Expression levels were normalized to the β-actin protein level. Values (mean ± SDs) were obtained from at least three independent experiments. *P < 0.05 and **P < 0.01 by one-way ANOVA with Tukey’s test. ANOVA: analysis of variance; SB: silibinin; SD: standard deviation.
Fig. 3. SB promotes mitochondrial function in human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells. Cells were stimulated in the presence or absence of SB at different doses for 24 h in a six-well plate. (A) Content of ATP in normal cells detected by the luminometric assay. (B) Effect of SB on MMP change in SB-treated normal cells. (C) The expression of mitochondrial mtDNA copy number determined by qPCR. (D) ROS levels in SB-treated normal cells detected by flow cytometry. Values (mean ± SDs) were obtained from at least three independent experiments. *P < 0.05 and **P < 0.01 by one-way ANOVA with Tukey’s test. ANOVA: analysis of variance; MMP: mitochondrial membrane potential; qPCR: quantitative polymerase chain reaction; ROS: reactive oxygen species; SB: silibinin; SD: standard deviation.
Fig. 4. SB promotes mitochondrial fission in human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells. (A) Cells treated with SB for 24 h were stained with MitoRed (red) and co-stained with DAPI (blue) to demonstrate mitochondrial morphology (red) and nucleus (blue) by fluorescence microscopy, respectively. (B, C) The protein and gene expression of mitochondrial fission in cells treated with SB at different doses for 24 h. (B) The protein level of Drp1 in cells. The mRNA level of Drp1 in cells. The Drp1 mRNA levels were normalized to β-actin gene levels. The protein and gene expression levels of Drp1 in cells were detected by western blotting and qPCR, respectively. Values (mean ± SDs) were obtained from at least three independent experiments. *P < 0.05 and **P < 0.01 by one-way ANOVA with Tukey’s test. ANOVA: analysis of variance; DAPI: 4',6-diamidino-2-phenylindole; SB: silybin; SD: standard deviation.
demonstrated that SB directly triggered the progression from G1 phase to S phase in normal cells, consequently promoting cells proliferation.

Cell cycle progression is promoted by the mitochondrial dynamic changes, especially in G1-to-S phase progression. Mitochondrial dynamic is important for keeping mtDNA...
Fig. 6. SB promotes mitochondrial dynamic process and G1 to S transition during the cell cycle in human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells by activating the Drp1-mediated mitochondrial fission pathway. Cells were transfected with Drp1-siRNA (100 nM) or negative control siRNA for 24 h prior SB treatment. (A) The blockage of Drp1 decreased the ratio of G1 to S transition during the cell cycle. The cell cycle was analyzed by flow cytometry. Values (mean ± SDs) were obtained from at least three independent experiments. *P < 0.05 and **P < 0.01, and ***P < 0.001, versus negative control siRNA group. #P < 0.05 and ##P < 0.01, versus SB treatment group by one-way ANOVA with Tukey's test. ANOVA: analysis of variance; SB: silibinin; SD: standard deviation.
distribution and preserving the integrity of mtDNA. With a unique giant and hyperfused network, mitochondria display higher ATP producing ability at the G1/S phase cell cycle. ATP, generated in mitochondria, has been considered as the most crucial single-molecule supplying energy in life. ROS production is correlated to the ATP producing ability in mitochondria. Mitochondrial fission plays an important role in keeping ROS levels in check. Moderate levels of ROS could promote cell proliferation and survival. MMP, generated by mitochondrial respiratory chains in mitochondrial inner membranes, depended on energy to be generated and maintained. MMP is utilized for importing protein into the mitochondria. Consistently, we observed a promoting effect of increasing mitochondrial fission on ATP production and ROS production in cells treated with SB. Moreover, we found that elevated mitochondrial fission promotes mitochondrial MMP and mtDNA copy number through upregulating Drp1 expression. Mitochondrial morphology and function are maintained through the mitochondrial dynamic. In mitochondrial dynamic, mitochondrial fission facilitates mitochondrial distribution and segregate damaged sections from mitochondria. Drp1, a cytosolic protein, is a major mediator in mitochondrial fission. With directly controlling mitochondrial morphology, Drp1 is crucial for cell proliferation, death, metabolism, and ROS production. Our study has shown that SB enhanced the expression of Drp1 and led to more mitochondrial fragmentation and fewer tubules in normal cells. Some main cell cycle mediators can directly regulate mitochondrial dynamics. During the regulated cell cycle, Drp1 has been demonstrated as a major mediator in cell cycle control. The major cyclins have functionally or molecularly links with Drp1 activity. In the previous study, we found that SB inhibited cervical cancer cell proliferation through inducing G2/M cell cycle arrest via activation of the Drp1. In this study, SB promoted normal cell proliferation through facilitating G1/S transition. The promotion of G1/S transition could accumulate the energy and biosynthetic capacity for a new round of proliferation, while the arrest of G2/M cell cycle inhibits the cell enters mitosis. Thus, to verify whether the SB-promoted G1/S phase in the normal cell cycle is mediated by Drp1, we investigated whether the downregulation of Drp1 could reduce SB-induced cell proliferation. In this study, knockdown of Drp1 decreased the cell colony number and proliferation, reduced the ratio of G1/S phase in the cell cycle and the expression of CDK2 and cyclinE1. These evidences indicated that SB-induced mitochondrial fission played a critical role in the key G1/S cell cycle transition, which contributed to normal cell proliferation. In the further study, we would like to focus on the specific mechanism of SB promoting the proliferation of normal cells and inhibiting the growth of tumor cells by mediating Drp1, to find a promising medicine for the treatment of cancer.

Conclusions

Our study illustrates that SB promotes G1/S cell cycle transition through Drp1-mediated mitochondrial fission, and thus promotes proliferation in human AC16 cardiomyocytes, LO2 hepatocytes, and human proximal tubular epithelial HK2 cells. Therefore, SB may be a potentially useful herbal derivative for the daily prevention and regeneration of various diseases caused by impaired mitochondrial fission.
Author Contributions
All the authors contributed sufficiently for their participation in the study, as follows: Xiaoshan Zhao and Jiaojiao Dai are conceived, designed, and supervised the study; Yanting You, Liqian Chen, Yifen Wu, Ming Wang, Hanqi Lu, and Xinghong Zhou performed cell research; Yanting You, Huaxi Liu, Zixuan Fu, and Quixing He did data analysis and interpretation; Yanting You, Liqian Chen, Yifen Wu, Jinying Ou, and Xiuqiong Fu wrote the paper; and Huanye Kwan, Yanyan Liu, and Donghui Liang modified the paper. All authors have read and approved the final manuscript.

Statement of Human and Animal Rights
This study did not include the human study and animal study.

Data Availability Statement
The datasets generated for this study are available on request to the corresponding author.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References
1. Luper S. A review of plants used in the treatment of liver disease: part 1. Altern Med Rev. 1998;3(6):410–421.
2. Loguercio C, Festi D. Silybin and the liver: from basic research to clinical practice. World J Gastroenterol. 2011;17(18):2288–2301.
3. Lee Y, Park HR, Chun HJ, Lee J. Silibinin prevents dopaminergic neuronal loss in a mouse model of Parkinson’s disease via mitochondrial stabilization. J Neurosci Res. 2015;93(5):755–765.
4. Barcena R, Moreno A, Rodriguez-Gandia MA, Albillos A, Arocena C, Blesa C, Garcia-Hoz F, Graus J, Nuno J, Lopez-Hervas P, Gajate L, et al. Safety and anti-HCV effect of prolonged intravenous silibinin in HCV genotype 1 subjects in the immediate liver transplant period. J Hepatol. 2013;58(3):421–426.
5. Luo H, Vong CT, Chen H, Gao Y, Lyu P, Qiu L, Zhao M, Liu Q, Cheng Z, Zou J, Yao P, et al. Naturally occurring anticancer compounds: shining from Chinese herbal medicine. Chin Med. 2019;14:48.
6. Straub SP, Stiller SB, Wiedemann N, Pfanner N. Dynamic organization of the mitochondrial protein import machinery. Biochim Biophys Acta. 2016;1861(11):1097–1114.
7. Lackner LL. Shaping the dynamic mitochondrial network. BMC Biol. 2014;12:35.
8. Eisner V, Picard M, Hajnoczky G. Mitochondrial dynamics in adaptive and maladaptive cellular stress responses. Nat Cell Biol. 2018;20(7):755–765.
9. Jhun BS, O-Uchi J, Adaniya SM, Cypress MW, Yoon Y. Adrenergic regulation of Drp1-driven mitochondrial fission in cardiac physio-pathology. Antioxidants (Basel). 2018;7(12):195.
10. Cho HM, Ryu JR, Jo Y, Seo TW, Choi YN, Kim JH, Chung JM, Cho B, Kang HC, Yu SW, Yoo SJ, et al. Drp1-Zip1 interaction regulates mitochondrial quality surveillance system. Mol Cell. 2019;73(2):364–376.
11. Hu Q, Zhang H, Gutierrez CN, Wu D, Wang P, Zhang J, Mattison JA, Smith E, Betcher LF, Wang M, Lakatta EG, et al. Increased Drp1 acetylation by lipid overload induces cardiomyocyte death and heart dysfunction. Circ Res. 2020;126(4):456–470.
12. Li S, Lin Q, Shao X, Zhu X, Wu J, Wu B, Zhang M, Zhou W, Zhou Y, Jin H, Zhang Z, et al. Drp1-regulated PARK2-dependent mitophagy protects against renal fibrosis in unilateral ureteral obstruction. Free Radic Biol Med. 2019;152:632–649.
13. Zhou H, Zhu P, Wang J, Toan S, Ren J. DNA-PKcs promotes alcohol-related liver disease by activating Drp1-related mitochondrial fission and repressing FUNDC1-required mitophagy. Sig Transduct Target Ther. 2019;4:56.
14. Lee YG, Nam Y, Shin KJ, Yoon S, Park WS, Joung JY, Seo JK, Jang J, Lee S, Nam D, Caino MC, et al. Androgen-induced expression of DRP1 regulates mitochondrial metabolic reprogramming in prostate cancer. Cancer Lett. 2020;471:72–87.
15. Favaro G, Romanello V, Varanita T, Andrea DM, Morbidoni V, Tezze C, Albiero M, Canato M, Gherardi G, De Stefani D, Mammucari C, et al. DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. Nat Commun. 2019;10(1):2576.
16. Gui C, Ren Y, Chen J, Wu X, Mao K, Li H, Yu H, Zou F, Li W. p38 MAPK-DRP1 signaling is involved in mitochondrial dysfunction and cell death in mutant A53 T alpha-synuclein model of Parkinson’s disease. Toxicol Appl Pharmacol. 2019;388:114874.
17. You Y, Zhang Y, Lu Y, Hu K, Qu X, Liu Y, Lu B, Jin L. Protein profiling and functional analysis of liver mitochondria from rats with nonalcoholic steatohepatitis. Mol Med Rep. 2017;16(3):2379–2388.
18. Song L, Liu T, Song Y, Sun Y, Li H, Xiao N, Xu H, Ge J, Bai C, Wen H, Zhang Y, et al. mtDNA copy number contributes to all-cause mortality of lacunar infarct in a Chinese prospective stroke population. J Cardiovasc Transl Res. 2019.
19. Maillet V, Boussetta N, Leclere J, Fauveau V, Foretz M, Viollet B, Couty JP, Celton-Morizur S, Perret C, Desdouets C. LKB1 as a gatekeeper of hepatocyte proliferation and genomic integrity during liver regeneration. Cell Rep. 2018;22(8):1994–2005.
20. Gunthel M, Barnett P, Christoffels VM. Development, proliferation, and growth of the mammalian heart. Mol Ther. 2018;26(7):1599–1609.
21. Chang-Panesso M, Kadyrov FF, Lalli M, Wu H, Ikeda S, Kefaloyianni E, Abdelmageed MM, Herrlich A, Kobayashi A, Humphreys BD. FOXM1 drives proximal tubule proliferation during repair from acute ischemic kidney injury. J Clin Invest. 2019;129(12):5501–5517.
22. Anestopoulos I, Kavo A, Tentes I, Kortsaris A, Panayiotidis M, Lazou A, Pappa A. Silibinin protects H9c2 cardiac cells from oxidative stress and inhibits phenylephrine-induced hypertrophy: potential mechanisms. J Nutr Biochem. 2013;24(3):586–594.
23. Zhang Y, Hai J, Cao M, Zhang Y, Pei S, Wang J, Zhang Q. Silibinin ameliorates steatosis and insulin resistance during non-alcoholic fatty liver disease development partly through targeting IRS-1/PI3K/Akt pathway. Int Immunopharmacol. 2013;17(3):714–720.
24. Dorsey S, Tollis S, Cheng J, Black L, Notley S, Tyers M, Royer CA. G1/S transcription factor copy number is a growth-dependent determinant of cell cycle commitment in yeast. Cell Syst. 2018;6(5):539–554.
25. Honda R, Lowe ED, Dubinina E, Skamnaki V, Cook A, Brown NR, Johnson LN. The structure of cyclin E1/CDK2: implications for CDK2 activation and CDK2-independent roles. Embo J. 2005;24(3):452–463.
26. Bao D, Zhao J, Zhou X, Yang Q, Chen Y, Zhu J, Yuan P, Yang J, Qin T, Wan S, Xing J. Mitochondrial fission-induced mtDNA stress promotes tumor-associated macrophage infiltration and HCC progression. Oncogene. 2019;38(25):5007–5020.
27. Mitra K, Wunder C, Roysam B, Lin G, Lippincott-Schwartz J. A hyperfused mitochondrial state achieved at G1-S regulates cyclin E buildup and entry into S phase. Proc Natl Acad Sci U S A. 2009;106(29):11960–11965.
28. Senos DR, Jones DL. Mitochondrial fission regulates germ cell differentiation by suppressing ROS-mediated activation of epidermal growth factor signaling in the Drosophila larval testis. Sci Rep. 2019;9(1):19695.
29. Li Q, Zhou T, Liu C, Wang XY, Zhang QJ, Wu F, Lin G, Ma YM, Ma BL. Mitochondrial membrane potential played crucial roles in the accumulation of berberine in HepG2 cells. Biosci Rep. 2019;39(4):BSR20190477.
30. Martinez-Reyes I, Diebold LP, Kong H, Schieber M, Huang H, Hensley CT, Mehta MM, Wang T, Santos JH, Woychik R, Dufour E, et al. TCA cycle and mitochondrial membrane potential are necessary for diverse biological functions. Mol Cell. 2016;61(2):199–209.
31. Chen L, Winger AJ, Knowlton AA. Mitochondrial dynamic changes in health and genetic diseases. Mol Biol Rep. 2014;41(11):7053–7062.
32. Simula L, Campanella M, Campello S. Targeting Drp1 and mitochondrial fission for therapeutic immune modulation. Pharmacol Res. 2019;146:104317.
33. Mitra K. Mitochondrial fission-fusion as an emerging key regulator of cell proliferation and differentiation. Bioessays. 2013;35(11):955–964.
34. You Y, He Q, Lu H, Zhou X, Chen L, Liu H, Lu Z, Liu D, Liu Y, Zuo D, Fu X, et al. Silibinin induces G2/M cell cycle arrest by activating Drp1-dependent mitochondrial fission in cervical cancer. Front Pharmacol. 2020;11:271.