Anti-Tumor and Anti-Diabetic Effects of Sarsasapogenin

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Abstract In this paper, the pharmacological effects and molecular mechanisms of sarsasapogenin, such as anti-oxidant, anti-inflammatory and anti-diabetic effects, are reviewed in order to provide a theoretical basis for the subsequent development and clinical application of sarsasapogenin.

Key words Sarsasapogenin, Anti-tumor, Anti-diabetic, Anti-inflammatory

1 Introduction

Anemarrhena asphodeloides Bunge, also known as Chimu, Lianmu, Yeliao and Dishen, mainly grows in Hebei, Shanxi and Heilongjiang, and is often used to clear away heat and purge fire, nourish yin for moistening dryness, and replenish the vital essence and remove heat. Sarsasapogenin (SAR), also known as Spirostan-3-ol, is a kind of steroidal saponins extracted from *A. asphodeloides* Bunge. It is white in appearance, its density and boiling point are (1.1 ± 0.1) g/cm^3 and (516.6 ± 20.0) ℃, respectively, and its molecular formula is $C_{27}H_{44}O_3$. It is easily soluble in organic solvents such as methanol, ethanol and DMSO, and has good anti-oxidant, anti-inflammatory and antibacterial effects[1]. Recent studies have shown that SAR can block cancer cell cycle and induce apoptosis by regulating MAPK and Akt signaling pathways in cancer cells[2]. In this paper, the pharmacological action and molecular mechanism of SAR are reviewed to provide a theoretical basis for its further application.

2 Anticancer effect of sarsasapogenin

Cancer is a disease caused by gene mutation or excessive proliferation of normal cells, having the characteristics of infinite proliferation, metastasis and invasiveness. At present, although clinical chemotherapy drugs have played a certain role in the treatment of cancer, there are still many problems such as strong adverse reactions, high drug resistance and high price. Therefore, it is extremely urgent to find a drug with low toxicity, safety, low drug resistance and low price. It has been found that SAR has the advantages of less toxic and side effects, short treatment period and low price, and is expected to become a new type of anti-tumor drug in clinic.

2.1 Anti-colorectal cancer effect and its molecular mechanism Colorectal cancer (CRC) is a kind of digestive tract malignant tumor with high morbidity and mortality, which originates from colonic mucosal epithelial cells. Wu Yuantao *et al.*[2] found by MTT assay and flow cytometry that SAR could effectively reduce the viability of colorectal cancer HT-29 cells and induce apoptosis (apoptosis rate of 14.36%). By Western Blotting and MDC staining, it was found that SAR could up-regulate the expression levels of Caspase-3, Caspase-9, Beclin-1 and LC3B, increase the fluorescence intensity of MDC, increase the autophagy activity of colorectal cancer HT-29 cells and induce apoptosis. Ling Bofan *et al.*[3-4] found that SAR could effectively reduce the viability and induce apoptosis of colorectal cancer LoVo cells by MTT assay and AnnexinV/PI double staining (apoptosis rate of 69.03%). By flow cytometry, it was found that SAR could induce LoVo cell cycle arrest in G_2/M phase (inhibition rate of 78.16%). Through Transwell test, it was found that SAR could reduce the invasion ability of cancer cells (inhibition rate of 35%). These results indicate that SAR can effectively inhibit the proliferation of colorectal cancer cells, reduce their adhesion and invasion ability, and induce apoptosis of cancer cells.

2.2 Anti-gastric cancer effect and its molecular mechanism Gastric cancer (GC) is one of the most common malignant tumors, having the characteristics of high morbidity, high mortality, high recurrence rate and high metastasis rate. The new cases in China account for about 42% of the total cases in the world every year[5]. Liao Zijun *et al.*[6] found by flow cytometry that SAR could effectively inhibit the proliferation of gastric cancer BGC-823 cells (inhibition rate of 83.75%) and induce their apoptosis (apoptosis rate of 39.43%). Xue Weiwei *et al.*[7] found that SAR could induce apoptosis of gastric cancer BGC-823 cells (apoptosis rate of 29.2%) and inhibit the invasion ability of cancer cells (inhibition rate of 35%) by Annexin V/PI double staining and Transwell test. These results indicate that SAR can effectively inhibit the proliferation and invasion of gastric cancer cells and induce their apoptosis.

2.3 Anti-hepatoma effect and its molecular mechanism Hepatocellular carcinoma (HCC) is a malignant tumor occurring in intrahepatic bile duct epithelial cells, with the characteristics of high incidence and strong concealment. Ni Yuan *et al.*[8] found that SAR could significantly reduce the viability and induce apoptosis of hepatoma HepG2 cells by MTT assay and flow cytometry. The IC_{50}, cycle inhibition rate and apoptosis rate of HepG2 cells treated with SAR were 25 μg/mL, 46.33% and 37.72%, respec-

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tively after 24 h. Furthermore, Western Blotting assay showed that SAR could down-regulate the expression of p-AKT (Thr308, Ser473) and up-regulate the expression of cytochrome C (Cyt c) and ROS, indicating that SAR could induce apoptosis of hepatoma cells by regulating PI3K/AKT signaling pathway. Bao W *et al.* [9] detected by Hoechst 33258 staining test that the apoptosis number of hepatoma HepG2 cells treated with SAR (50 μg/mL) for 72 h was significantly larger than that of the control group. By electron microscopy and DNA fragment analysis, it was found that the number of apoptotic bodies in HepG2 cytoplasm after SAR treatment increased, and obvious apoptosis characteristics such as DNA fragmentation, chromatin aggregation and cell contraction appeared at the same time. These results indicate that SAR can effectively reduce the viability and proliferation of HepG2 cells, and promote the decrease of mitochondrial membrane potential, thus inducing apoptosis of hepatoma cells.

2.4 Anti-ovarian cancer effect and its molecular mechanism Ovarian cancer (OC) originates in fallopian tube and peritoneum, as a malignant tumor related to heredity, hormone and other factors. Guo Hong *et al.* [10] found that SAR could significantly inhibit the proliferation (inhibition rate of 63.79%), colony formation number, migration distance and invasive cell number of ovarian cancer CAOV3 cells by MTT assay, plate cloning test, scratch test and Transwell test. Furthermore, real-time quantitative PCR (RT-qPCR) and Western Blotting assay showed that SAR could down-regulate the expression level of circ-PRKCI RNA and up-regulate the expression level of miR-130a-5p, and then down-regulate the expression level of E-cadherin and up-regulate the expression level of N-cadherin, and finally inhibit the proliferation, invasion and migration of ovarian cancer CAOV3 cells. These results suggest that SAR can regulate the expression of circ-PRKCI/miR-130a-5p, reduce the viability of ovarian cancer CAOV3 cells, inhibit the proliferation, migration and invasion of cancer cells, and induce apoptosis of ovarian cancer cells.

2.5 Anti-cervical cancer effect and its molecular mechanism Cervical cancer is a common gynecological malignant tumor originating from cervix. Shen S *et al.* [11] found by MTT assay and flow cytometry that SAR could effectively reduce the viability of cervical cancer HeLa cells, increase the number of cancer cells in G2/M phase, and change the mitochondrial membrane potential of cancer cells. By Hoechst staining, it was found that SAR significantly increased the apoptosis rate of HeLa cells (84.33%) compared with the control group. Furthermore, Western Blotting assay showed that SAR could increase the ratio of pro-apoptosis protein Bax to anti-apoptosis protein Bcl-2, up-regulate the expression levels of Caspase-9, Caspase-3 and cytochrome C (Cyt c), and activate UPR signaling pathway by up-regulating the expression level of ROS, thus inducing endoplasmic reticulum (ER) stress and apoptosis of cervical cancer HeLa cells. These results suggest that SAR can block the cell cycle and induce ER stress and mitochondrial-dependent apoptosis by up-regulating ROS expression.

3 Anti-diabetic effect

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia caused by insulin secretion deficiency or impaired biological function. It is mainly divided into two types: type I diabetes mellitus and type II diabetes mellitus, and its main symptoms include polyuria, polyphagia, polydipsia and weight loss[12]. Long-term hyperglycemia can cause complications of many organs and systems, such as Alzheimer's disease, slow inflammation, nephropathy and podocytosis[13].

3.1 Anti-diabetic Alzheimer's disease effect and its molecular mechanism Alzheimer's disease (AD) is a common neurodegenerative disease in the elderly, which is mainly characterized by memory impairment and cognitive dysfunction. AD is related to diabetes in epidemiology, histopathology and molecular biochemical characteristics, and type 2 diabetes can significantly increase the probability of AD[14-15]. Zhang YM *et al.* [16] found through Morris Water Maze (MWM) experiment that compared with the model group, the number of diabetic rats treated with SAR increased significantly in the space platform and the time spent in the target quadrant, indicating that SAR had neuroprotective effect and could improve the learning and memory impairment of rats. Immunofluorescence assay and Western Blotting assay showed that SAR could up-regulate the expression of PPAR γ in human neuroblastoma SH-SY5Y cells, activate PPAR γ signaling pathway, inhibit the overexpression of BACE1/Aβ, and down-regulate the phosphorylation level of microtubule-associated (tau) protein in hippocampus of diabetic rats, thus reducing the loss of neurons in hippocampus of diabetic rats, protecting nerves and delaying the development of Alzheimer's disease caused by diabetes.

3.2 Anti-diabetic nephropathy effect and its molecular mechanism Diabetic nephropathy (DKD) is one of the irreversible microvascular complications caused by long-term high glucose or abnormal glucose metabolism in patients, which will lead to abnormal renal function (proteinuria, decreased glomerular filtration rate) and even death from renal failure[17]. Tang ZZ *et al.* [18] used streptozotocin (STZ) to construct diabetic rat model. After SAR (60 mg/kg) treatment, it was found that SAR could effectively reduce the levels of creatinine, urea nitrogen and uric acid in rats, as well as the thickness of glomerular basement membrane, and inhibit the proliferation of glomerular mesangial cells (HMCs). By Western Blotting, immunofluorescence staining and RT-qPCR, it was found that SAR could down-regulate the expression of PAR-1 mRNA in HMC cells, and inhibit the activation of NLRP3 inflammatory corpuscle and NF-κB signaling pathway, thus alleviating the nephropathy caused by diabetes. LI XZ *et al.* [19] found that SAR could effectively reduce the levels of blood glucose, triglyceride and total cholesterol in serum of diabetic rats, and inhibit podocyte fusion and renal fibrosis. Through network pharmacological analysis, it was found that GSK-3β was a potential target for SAR to treat DKD. Further, Western Blotting test showed that SAR could indeed down-regulate the expression level of GSK-3β and inhibit the activation

of AMPK and mTOR signaling pathways mediated by SAR, thus improving the damage degree of podocytes and reducing their autophagy level.

4 Prospects

Sarsasapogenin, as a saponin compound in *A. asphodeloides* Bunge, can effectively inhibit the proliferation, migration and invasion of tumor cells and induce apoptosis of tumor cells, with good anti-tumor effect. In addition, it also has the advantages of abundant sources, less toxic and side effects, easy preparation and low price. However, sarsasapogenin is a traditional Chinese medicine compound, and the research and clinical application of its specific pharmacological activity and molecular mechanism is still in the primary stage. It is necessary to continuously continuously combine the relevant theories and experimental techniques of molecular biology, cell biology, experimental zoology, pharmacology and basic medicine to conduct a more comprehensive and in-depth study of sarsasapogenin from the molecular, cellular and animal levels, so as to lay a foundation for the further research, development and utilization of sarsasapogenin.

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(From page 58)

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