Anti-tumor Effects of Morusin Exerted by Inducing Apoptosis

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Abstract Morusin is a flavonoid compound isolated and extracted from the root bark of *Morus alba* L. Studies have reported that morusin exerts anti-tumor effects by inhibiting cancer cell invasion and proliferation, as well as inducing tumor cell apoptosis. This article comprehensively reviews recent research on the anti-tumor effects of morusin and its related molecular mechanisms, aiming to provide theoretical support for further studies and new drug development of morusin.

Key words Morusin, Anti-tumor, Cell proliferation, Apoptosis, Cell cycle arrest

1 Introduction

Morus alba L., also known as "Sanggen Baipi", is primarily distributed in Anhui, Henan, Zhejiang, Jiangsu, and Hunan provinces. It is traditionally used to alleviate symptoms such as lung heat-induced cough, edema with oliguria, and facial or skin puffiness[1]. Morusin, also known as sanggenin, is a flavonoid compound extracted and isolated from *M. alba* L. It appears as a pale yellow powder with the molecular formula $C_{25}H_{24}O_6$ and is readily soluble in DMSO and hot methanol. Studies have shown that morusin exerts anti-tumor effects by inhibiting cancer cell invasion and proliferation, as well as inducing tumor cell apoptosis. This article reviews recent research on the anti-tumor effects of morusin and its molecular mechanisms, providing a theoretical basis for further exploration and utilization of morusin.

2 Anti-tumor effects of morusin and molecular mechanisms

2.1 Anti-tumor effects on pancreatic cancer and molecular mechanisms Pancreatic cancer is a malignant tumor originating from pancreatic ductal epithelial and acinar cells, characterized by rapid progression, low surgical resection rates, and extremely high mortality. Sun Wenli[2] investigated the inhibitory effects of morusin on the proliferation of pancreatic cancer AsPC-1 and HPAC cells using the MTT assay. The results showed that morusin suppressed the proliferation of AsPC-1 and HPAC cells in a concentration-dependent manner (0.5, 5, 10, 15, 20, 25, 30, 35, and 40 μM). Flow cytometry was used to examine the effects of morusin on cell cycle progression in AsPC-1 and HPAC cells. The results revealed that increasing concentrations of morusin gradually increased the proportion of AsPC-1 and HPAC cells arrested in the G_0/G_1 phase. Western blot analysis was further employed to assess the impact of morusin on the expression levels of cell cycle-related proteins in AsPC-1 and HPAC cells. The results demonstrated that morusin treatment significantly upregulated the protein expression levels of Cyclin D1, CDK4, and CDK6 in AsPC-1 and HPAC cells, indicating that morusin inhibits the proliferation of pancreatic cancer cells by regulating cell cycle-related signaling pathways.

2.2 Anti-tumor effects on glioma and molecular mechanisms Glioma is a neuroepithelial tumor originating from the central nervous system, characterized by high invasiveness and malignancy. Tang Dong *et al.*[3] evaluated the inhibitory effects of morusin on the proliferation of glioma U87 cells using the CCK-8 assay. The results showed that morusin inhibited the proliferation of U87 cells in a concentration-dependent manner. The inhibitory effects of morusin on U87 cell migration were examined using scratch wound healing and Transwell assays. The results indicated that morusin treatment significantly reduced the scratch healing rate and invasiveness of U87 cells. Western blot analysis was further conducted to investigate the effects of morusin on the expression levels of migration- and invasion-related proteins in U87 cells. The results demonstrated that morusin treatment significantly upregulated vimentin expression and downregulated MMP-2 protein levels in U87 cells, suggesting that morusin reduces glioma cell viability by suppressing proliferation and migration.

Yang Lu *et al.*[4] assessed the inhibitory effects of morusin on the proliferation of glioma U251 cells using the CCK-8 assay. The results showed that morusin inhibited U251 cell proliferation in both concentration-dependent (1, 10, 100, and 1 000 μg/mL) and time-dependent (24, 48, 72, and 96 h) manners. The Transwell assay was used to evaluate the inhibitory effects of morusin on U251 cell migration. The results revealed that increasing concentrations of morusin progressively reduced the invasiveness of U251 cells. Western blot analysis was further performed to examine the effects of morusin on the expression levels of proliferation-related proteins in U251 cells. The results demonstrated that morusin treatment significantly increased the phosphorylation levels of Wnt/β-catenin, Cyclin D1, and β-catenin in U251 cells, indicating that morusin suppresses glioma cell proliferation by modulating the Wnt/β-catenin signaling pathway.

2.3 Anti-tumor effects on papillary thyroid carcinoma and molecular mechanisms Papillary thyroid carcinoma (PTC) is

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the most common type of thyroid cancer, accounting for 85% of all thyroid malignancies[5]. Fan Hao *et al.*[6] investigated the inhibitory effects of morusin on the proliferation of papillary thyroid carcinoma TPC-1 cells using the MTT assay. The results showed that morusin inhibited the proliferation of TPC-1 cells in a concentration-dependent manner (0, 2.5, 5, 10, 20, 40, 80, and 80 μg/L). Hoechst/PI staining was used to assess the apoptosis-inducing effects of morusin on TPC-1 cells. The results revealed increased membrane permeability in TPC-1 cells, along with a gradual increase in cells displaying condensed and densely stained nuclei, indicating morphological changes characteristic of apoptosis. Flow cytometry was employed to evaluate the apoptosis-inducing effects of morusin on TPC-1 cells. The results demonstrated that morusin treatment significantly increased the apoptosis rate of TPC-1 cells. Western blot analysis was further conducted to examine the effects of morusin on the expression levels of apoptosis-related proteins in TPC-1 cells. The results showed that morusin treatment significantly upregulated the expression levels of Bax, p38/MAPK, p53, and PUMA, while downregulating MDM2 protein levels in TPC-1 cells. This indicates that morusin induces apoptosis in papillary thyroid carcinoma cells by modulating the P38/MAPK-MDM2-P53 signaling pathway.

2.4 Anti-tumor effects on gastric cancer and molecular mechanisms Gastric cancer is one of the most common malignant tumors, with China accounting for 42% of global new cases annually[7]. Wang Feng[8] evaluated the inhibitory effects of morusin on the proliferation of gastric cancer MKN45 and SGC7901 cells using the MTT assay. The results demonstrated that morusin suppressed the proliferation of MKN45 and SGC7901 cells in a concentration-dependent manner (1, 2, and 5 mg/L). Flow cytometry was used to analyze the effects of morusin on cell cycle progression in MKN45 and SGC7901 cells. The results revealed that increasing concentrations of morusin reduced the proportion of cells in the S phase and increased the proportion in the G_0/G_1 phase, indicating that morusin induces G_0/G_1 phase arrest in gastric cancer cells. Western blot analysis was further performed to assess the effects of morusin on the expression of cell cycle-related proteins in MKN45 and SGC7901 cells. The results showed that morusin treatment significantly downregulated Cyclin D1 expression in these cells, suggesting that morusin inhibits gastric cancer cell proliferation via cell cycle arrest.

2.5 Anti-tumor effects on multiple myeloma and molecular mechanisms Multiple myeloma is a common hematologic malignancy originating from plasma cells in the bone marrow[9]. Li Caiyu[10] investigated the inhibitory effects of morusin on the proliferation of myeloma ARP-1 cells using the CCK-8 assay. The results showed that morusin inhibited ARP-1 cell proliferation in both concentration-dependent (0, 3, 6, 9, and 12 μmol/L) and time-dependent (12, 24, and 36 h) manners. Flow cytometry was used to evaluate the apoptosis-inducing effects of morusin on ARP-1 cells. The results demonstrated that morusin treatment significantly increased the apoptosis rate of ARP-1 cells. Western blot and RT-qPCR analyses were performed to examine the effects of morusin on the expression levels of apoptosis-related proteins and mRNAs in ARP-1 cells. The results revealed that morusin treatment significantly upregulated Caspase-3, PTEN, and Bax expression, while downregulating PI3K, AKT1, and Bcl-2 levels in ARP-1 cells. This indicates that morusin suppresses myeloma cell proliferation by modulating the PI3K/AKT signaling pathway.

2.6 Anti-tumor effects on melanoma and molecular mechanisms Melanoma is an aggressive skin cancer originating from melanocytes in the skin, eyes, or mucous membranes. Liu Wei *et al.*[11] assessed the inhibitory effects of morusin on the proliferation of melanoma A375 and MV3 cells using the MTT assay. The results showed that morusin inhibited the proliferation of A375 and MV3 cells in a concentration-dependent manner (0.001, 0.01, 0.1, 1, 10, 20, 40, 60, 80, and 160 μM). The scratch wound healing and Transwell assays were used to evaluate the inhibitory effects of morusin on the migration and invasion of A375 and MV3 cells. The results revealed that increasing concentrations of morusin progressively reduced the migration rate and invasive capacity of A375 and MV3 cells. Flow cytometry was employed to analyze the effects of morusin on the cell cycle progression of A375 and MV3 cells. The results demonstrated that morusin treatment increased the proportion of A375 and MV3 cells arrested in the G2/M phase. Western blot analysis was further conducted to examine the effects of morusin on the expression levels of apoptosis-related proteins in A375 and MV3 cells. The results showed that morusin treatment significantly upregulated the expression levels of Cleaved-PARP, Cleaved-Caspase3, p-JNK, and p-ERK in these cells, indicating that morusin induces apoptosis in melanoma cells by regulating apoptosis-related protein expression.

2.7 Anti-tumor effects on nasopharyngeal carcinoma and molecular mechanisms Nasopharyngeal carcinoma (NPC) is an epithelial malignancy originating from the mucosal lining of the nasopharynx, predominantly occurring in the roof and lateral walls. Guo Xingzhe[12] investigated the inhibitory effects of morusin on the proliferation of nasopharyngeal carcinoma 5-8F and CNE2 cells using the CCK-8 assay. The results demonstrated that morusin suppressed the proliferation of 5-8F and CNE2 cells in a concentration-dependent manner (0, 10, 20, 30, 40, 50, 60, 70, and 80 μmol/L). Scratch wound healing and Transwell assays were used to evaluate the effects of morusin on the migration and invasion of 5-8F and CNE2 cells. The results showed that increasing concentrations of morusin significantly reduced the scratch healing rate and invasive capacity of 5-8F and CNE2 cells. Flow cytometry was employed to assess the apoptosis-inducing effects of morusin on 5-8F and CNE2 cells. The results revealed that morusin treatment significantly increased the apoptosis rate of these cells. Western blot analysis was further performed to examine the effects of morusin on the expression levels of apoptosis-related proteins in 5-8F and CNE2 cells. The results demonstrated that morusin treat-

ment significantly upregulated Bax expression and downregulated Bcl-2, p-ERK, p-PI3K, and p-Akt levels in these cells, suggesting that morusin inhibits nasopharyngeal carcinoma cell proliferation by modulating the ERK/PI3K/Akt signaling pathway.

2.8 Anti-tumor effects on non-small cell lung cancer and molecular mechanisms Non-small cell lung cancer (NSCLC) is a malignant tumor originating from bronchial mucosa or glands, and it is one of the most prevalent and lethal malignancies in China. Wang Jinxia[13] investigated the inhibitory effects of morusin on the proliferation of NSCLC A549 and NCI-H292 cells using the MTT assay. The results showed that morusin suppressed the proliferation of A549 and NCI-H292 cells in both concentration-dependent (2.5, 5, 10, 20, 30, and 40 μM) and time-dependent (24, 48, and 72 h) manners. Flow cytometry was used to evaluate the apoptosis-inducing effects of morusin on A549 and NCI-H292 cells. The results demonstrated that morusin treatment significantly increased the apoptosis rate of these cells. Western blot analysis was further performed to examine the effects of morusin on the expression levels of apoptosis-related proteins in A549 and NCI-H292 cells. The results revealed that morusin treatment significantly upregulated PARP, Caspase-3, p-JNK, and p-ERK expression, while downregulating AKT protein levels. This indicates that morusin induces apoptosis in NSCLC cells by modulating the PI3K/AKT/ERK/JNK signaling pathway.

2.9 Anti-tumor effects on renal cell carcinoma and molecular mechanisms Renal cell carcinoma (RCC) is a kidney malignancy primarily originating from renal tubular epithelial cells, accounting for the majority of kidney tumors. Yang Chengfei *et al.*[14] assessed the inhibitory effects of morusin on the proliferation of RCC 769-P, 786-O, and OSRC-2 cells using the CCK-8 assay. The results demonstrated that morusin inhibited the proliferation of these cells in a concentration-dependent manner (2, 4, and 6 μg/mL). Flow cytometry was employed to analyze the effects of morusin on cell cycle progression in 769-P, 786-O, and OSRC-2 cells. The results showed that after treatment with morusin, the number of cells in the S phase decreased and the number of cells in the G_0/G_1 phase increased in the 769-P, 786-O and OSRC-2 cell cycle, indicating that morusin arrests the cycles of 769-P, 786-O and OSRC-2 cells in the G_0/G_1 phase. Western blot analysis was further conducted to evaluate the effects of morusin on the expression of cell cycle-related proteins in these cells. The results showed that after treatment with morusin, the expression levels of Cyclin D1 and Cyclin D2 in 769-P, 786-O and OSRC-2 cells were significantly down-regulated, indicating that morusin inhibits the proliferation of renal cell carcinoma 769-P, 786-O and OSRC-2 cells by regulating cycle-related signaling pathways.

2.10 Anti-tumor effects on prostate cancer and molecular mechanisms Prostate cancer is a malignant tumor occurring in the peripheral zone of the prostate, ranking as the second most common cancer in males. Ja Il Koo *et al.*[15] investigated the inhibitory effects of morusin on the proliferation of prostate cancer PC-3 and DU145 cells using the MTT assay. The results showed that morusin suppressed the proliferation of PC-3 and DU145 cells in a concentration-dependent manner (0, 5, and 10 μM). Flow cytometry was used to examine the effects of morusin on cell cycle progression in these cells. The results showed that after treatment with morusin, the number of cells in the S phase decreased and the number of cells in the G_0/G_1 phase increased, indicating that morusin arrests the cycles of PC-3 and DU145 cells in the G_0/G_1 phase. Western blot analysis was further performed to assess the effects of morusin on apoptosis-related protein expression in PC-3 and DU145 cells. The results demonstrated that morusin treatment downregulated HK2, LDH, PKM2, c-Myc, and FOXM1 expression, while upregulating PARP and Caspase-3 levels. This suggests that morusin induces apoptosis in prostate cancer cells PC-3 and DU145 by modulating apoptosis-related proteins.

2.11 Anti-tumor effects on cervical cancer and molecular mechanisms Cervical cancer is a common gynecological malignancy originating from the cervix. Wang Li *et al.*[16] evaluated the inhibitory effects of morusin on the proliferation of cervical cancer HeLa cells using the MTT assay. The results showed that morusin inhibited HeLa cell proliferation in a concentration-dependent manner (0.25, 0.5, 1, 2, and 4 μM). DAPI staining was used to assess the apoptosis-inducing effects of morusin on HeLa cells. The results revealed chromatin condensation and apoptotic body formation in HeLa cells. Western blot analysis was further conducted to examine the effects of morusin on apoptosis-related protein expression in HeLa cells. The results demonstrated that morusin treatment significantly downregulated NF-κB and Bcl-2 expression, while upregulating Bax and Caspase-3 levels. This indicates that morusin inhibits cervical cancer cell HeLa proliferation by modulating the NF-κB signaling pathway.

3 Perspectives

As a flavonoid compound isolated from *M. alba* L., morusin effectively inhibits tumor cell proliferation, migration, invasion, and induces apoptosis, demonstrating significant anti-tumor potential. Additionally, morusin exhibits advantages such as low toxicity, ease of preparation, and cost-effectiveness. However, morusin is a traditional Chinese medicine compound, and the research on its pharmacodynamic molecular mechanisms and clinical applications remains in the early stages. Further comprehensive studies integrating molecular biology, cell biology, experimental animal science, pharmacology, and basic medical theories and techniques are essential to exploring morusin at molecular, cellular, and animal levels, laying the foundation for its development and utilization.

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