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Research Progress in the Mechanism of Apoptosis and Autophagy on Hepatocarcinogenesis

Wang Xiaofeng[1], Gou Yulei[1], Chu Jingli[2], Li Tao[1], Shi Xiaoyan[1,3], Li Cuijuan[1,3], Guo Dongyan[4], Fan Yu[1,3,*]

[1]School of Basic Medicine, Shaanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, China
[2]The First Clinical Medical College of Shaanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, China
[3]Shaanxi Key Laboratory of Traditional Chinese Medicine System and Disease Prevention and Treatment, Xianyang, Shaanxi, China
[4]School of Pharmacy, Shaanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, China
[*]Corresponding author

Abstract: *Hepatocellular carcinoma (HCC) is a common malignant tumor. Apoptosis and autophagy play an important role in the occurrence and development of HCC.It has been found that there is an interaction between apoptosis and autophagy in the treatment of hepatocellular carcinoma. Apoptosis is one of the main ways of cell death in hepatocellular carcinoma. Cell autophagy in hepatocellular carcinoma has a bilateral role of protective autophagy and lethal autophagy. Apoptosis is one of the main ways of cell death in hepatocellular carcinoma. Cell autophagy in hepatocellular carcinoma has a bilateral role of protective autophagy and lethal autophagy. Protective autophagy can inhibit the apoptosis of liver cancer cells induced by anticancer drugs, while lethal autophagy can induce the death of liver cancer cells in cooperation with apoptosis. Therefore, clarifying the interaction between apoptosis and autophagy in liver cancer will help to improve the efficacy of anti-tumor drugs in the clinical treatment of liver cancer. This article mainly discusses the relationship between hepatocellular carcinoma cell apoptosis and autophagy and its related influencing factors.*

Keywords: *Apoptosis, Cell autophagy, Liver cancer, Synergy, Antagonism*

1. Introduction

Liver cancer is one of the major malignant tumors that seriously endangers human physical and mental health, and its survival time of patients is often only 6-9 months, of which primary liver cancer is the most common hepatocellular carcinoma, ranking third as the cause of cancer death in malignant tumors worldwide, ranking fifth in the incidence of malignant tumors in China, and the fatality rate is second, second only to lung cancer [1][2]. At present, the commonly used methods for the treatment of liver cancer in clinical practice are conventional chemotherapy, surgical resection, radiofrequency ablation, transcatheter arterial chemotherapy and liver transplantation, but the therapeutic effect is often not satisfactory due to its high frequency of recurrence and low objective response rate[3]. It has been found that immunity to apoptosis may be one of the important mechanisms of tumor escape. Autophagy has a complex bidirectional regulatory effect in tumors and is able to inhibit the initiation of tumorigenesis at the early stage of tumorigenesi[4], and material and energy balance to maintain growth and reproduction can be obtained by autophagic degradation during tumor growth[5]. Apoptosis and autophagy as the main mechanism of programmed cell death are closely related to the occurrence and development of liver cancer, and they have a complex interaction in liver cancer. Therefore, elucidating the relationship between apoptosis and autophagy in the development of liver cancer is an urgent problem to be solved to improve the efficacy of liver cancer. In this paper, we review the interaction between apoptosis and autophagy in liver cancer.

2. Mechanism of Apoptosis in Liver Cancer

Apoptosis is programmed cell death characterized by blebbing of the plasma membrane, cell shrinkage, nuclear fragmentation, chromatin condensation, and fragmentation of chromosomal

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DNA.Intrinsic apoptosis pathway and extrinsic apoptosis pathway are two currently recognized mechanisms of apoptosis. Endogenous apoptosis is mainly caused by intracellular stimuli such as DNA damage and oxidative stress causing Bcl-2 family proteins Bad, Bak, Bax and Bcl-2 to insert into the outer mitochondrial membrane voids, promoting cytochrome c release and sequentially activating caspase-3, 6, and 7 to induce apoptosis after forming an apoptotic complex of the precursor caspase-9/cytochrome c/apoptotic protease activator Apaf1.The extrinsic apoptotic pathway is assembled by a variety of death ligands, such as tumor necrosis factor-related apoptosis-inducing ligands TRAIL and TNF-α, Fas ligands, and death-inducing signaling complex (DISC) after binding to death receptors. DISC is composed of Fas-associated death domain (FADD) protein and procaspase-8,10. DISC can either activate downstream effectors caspase-3, 6, and 7 to directly induce cell death or indirectly promote cell death by activating the mitochondria-mediated intrinsic apoptotic pathway after cutting Bid, a Bcl-2 family member, into tBid[6].In the development of liver cancer, apoptosis can mediate cell death through both intrinsic and extrinsic apoptotic pathways.

3. Mechanism of autophagy in liver cancer

It has been found that autophagy plays different roles in different stages of liver cancer.First, autophagy is able to prevent tumor transformation by removing damaged organelles and specific proteins from normal cells and can also use tumor suppressor effects to suppress the inflammatory response and genomic instability of cancer cells[7].Second, proper autophagy in molding tumor cells can further promote the survival and deterioration of tumor cells and exacerbate the continuous deterioration of tumors by meeting their metabolic needs[8].

Typically, when cells receive autophagy-inducing signals, the upstream gene AMPK is activated, which promotes ULK1/ATG13/FIP200 complex formation by directly promoting or indirectly inhibiting mTOR to promote Beclin1 complex expression to initiate autophagy, LC3-I, catalyzed by the ATG5/ATG12/ATG16L complex, forms LC3-II rivets on the autophagosome membrane, followed by transporters such as p62 and NBR1 that transport unwanted organelles, misfolded proteins, etc. into the autophagosome to bind to lysosomes and enzymatically degrade autophagosome contents, a process known as autophagy.Among autophagy, mitophagy is the most common autophagy that removes organelles.Parkin-dependent mitophagy transports PINK1-induced ubiquitinated material into autophagosome membranes mainly via transporters; Parkin-independent mitophagy transports it into autophagosome membranes mainly via Bnip3 and NIX[9].In addition, it has been found that PI3K/Akt/mTOR signaling pathway can inhibit the occurrence of autophagy by promoting Akt and then promoting downstream mTOR, and PTEN can promote the occurrence of autophagy by inhibiting Akt and then inhibiting mTOR[10].However, in the development of liver cancer, autophagy still plays a role in promoting cell death or protecting cell survival through the above autophagy signaling pathways.

4. Interactive mechanism of apoptosis and autophagy in liver cancer

Programmed cell death is an innate self-regulatory mechanism of the body, which can be divided into apoptosis, autophagy, apoptosis, mitotic catastrophe and oncosis according to the mechanism of occurrence.It has been shown that apoptosis and autophagy can occur in the same cell, and autophagy often precedes apoptosi[11].Whether autophagy induces or inhibits apoptosis depends on factors such as the type and nature of the cell and the duration of stimulation/stress[12].P53 inactivation has been found to inhibit autophagy through the AMPK signaling pathway, which is associated with p53 activation inducing the development of autophagy in cells[13]; p53 overexpression can lead to AMPK dephosphorylation and LC3-II transformation, suggesting that p53 may inhibit the development of autophagy through the AMPK signaling pathway[14].Thus, it was shown that tumor suppressor p53 could promote or inhibit the development of autophagy by regulating AMPK.The extrinsic apoptosis pathway can be initiated by Gulin3 E3 ligase-mediated polyubiquitination and Fas receptor-FADD-procaspase-8 death-inducing signaling complex (DISC)[15].It has been found that the active autophagosome membrane of LC3 is an important platform for caspase-8 aggregation and activation, while loss of autophagy-related protein p62 prevents TRAIL-mediated apoptosis, and caspase-8 can cleave p62 to its loss and induce autophagic degradation when the death receptor is activated[16].In addition, the anti-apoptotic protein Bcl-2 can inhibit the development of autophagy by binding the autophagy-related protein Beclin1; the autophagy-related stress complex ATG5-ATG12 acts directly with FADD to trigger apoptosis-induced cell death[17].

It can be seen that apoptosis and autophagy interact in the development of liver cancer and the

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relationship is complex, and a variety of signal transduction pathways and regulators are involved. Current studies have found synergistic and antagonistic relationships between apoptosis and autophagy. The two roles of apoptosis and autophagy in the development of liver cancer are summarized as follows.

4.1 Synergistic effects of apoptosis and autophagy in liver cancer

Based on the theory that there is an interactive mechanism between apoptosis and autophagy in HCC, our group found that curcumin can promote both apoptosis and autophagy in HCC, and further found through p53 inhibitors and AMPK inhibitors that whether apoptosis or autophagy is inhibited, the occurrence of the other of the two is inhibited.Showed that curcumin could inhibit the proliferation of HCC cells through the synergistic effect of autophagic cell death (ACD) and apoptosis during curcumin treatment of HCC[18].Dihydromyricetin (DHM) is a flavonoid extracted from grape stems and leaves, which has anti-tumor and antioxidant functions.In recent years, studies have shown that DHM has a significant effect on liver injury and liver cancer.DHM can promote apoptosis of HCC cells by enhancing TP53 expression and phosphorylation at Ser15, while it can regulate upstream signaling pathways such as AMPK and ERK1/2 by inhibiting mTOR activation, thereby stimulating autolysosome overaccumulation to induce autophagy[19].Xia et al.[20] found that DHM could not only induce autophagy, but also significantly up-regulate P53 protein expression, activate Bax and Bak, inhibit Bcl2 expression, and then activate caspase-3 in four types of HCC cells (HepG2, QGY7701, Hepal-6 and MHcc97L), and finally lead to apoptosis of HCC cells, indicating that DHM has a synergistic effect in promoting apoptosis and inducing autophagy in HCC cells.B-Thujaplicin is now used in some health products and clinical adjuvant drugs.Zhang et al.[21]found that β-Thujaplicin could induce autophagy in HCC cells, and β-Thujaplicin could induce the expression of ROS when HepG2 cells were treated with β-Thujaplicin combined with autophagy blockers or agonists, followed by ACD mediated by inhibition of the Akt-mTOR signaling pathway.B-Thujaplicin was also able to induce apoptosis in HepG2 cells through the mitochondrial pathway.Apoptin is a tumor-specific pro-apoptotic protein. Li et al. [22]found that apoptin can not only promote apoptosis of hepatocellular carcinoma cells, but also promote mitophagy and inhibit the proliferation of hepatocellular carcinoma cells through the increase of NIX protein expression.With the development of apoptosis and autophagy, apoptin can significantly increase the expression of ROS in cells, while when ROS is inhibited, both apoptosis and autophagy are inhibited, indicating that apoptosis and autophagy play a synergistic role in promoting apoptin[23].

Alpha-fetoprotein (AFP) is well-known as a specific tumor marker for the diagnosis and prognosis of hepatocellular carcinoma, and serum AFP levels are elevated in up to 70% of HCC patients.It has been found that AFP, which is significantly elevated in serum, can directly interact with amino acid residues Glu-248, Asp-253 and His-257 of caspase-3 molecule in the cytoplasm and block caspases signaling cascade to inhibit hepatoma cell apoptosis[24][25].It has also been reported[26]that inhibition of AFP gene can enhance the cytotoxicity of therapeutic agents on AFP-positive HCC cells by activating HuR-mediated Fas/FADD apoptotic signaling.Wang et al.[27]found that the interaction between AFP and PTEN could activate the PI3K/Akt/mTOR signaling pathway to inhibit autophagy in HCC cells aggravating the disease, and the above AFP studies indicated that AFP could promote the aggravation of HCC by inhibiting apoptosis and autophagy.

4.2 Antagonism of apoptosis and autophagy in liver cancer

It has been reported that overexpressed cathepsin B (CtsB), CtsD, CtsL, CtsS and CtsZ are closely related to poor prognosis of liver cancer.The reduction of lysosomal CstB or D can weaken lysosomal protease hydrolysis, thereby impairing autophagic flux in hepatoma cells resulting in inhibition of autophagy and promoting cell death.However, lysosomal membrane permeabilization can lead to leakage of CtsB into the cytoplasm and promote hepatoma cell apoptosis by cleaving Bid into tBid[28].Cardiac glycosides (PPM) simultaneously activated lethal apoptosis and protective autophagy in HepG2 cells, whereas initiation of autophagy counteracted the inherent pro-apoptotic ability and weakened anticancer effects.Further studies revealed that the combination of PPM with autophagy inhibitors enhanced the pro-apoptotic activity and in vivo anti-tumor activity of PPM[29].Apigenin is a dietary flavonoid, which can have antioxidant and antitumor effects on a variety of cancers by promoting cell death and inducing cell cycle arrest.Yang et al.[30]found that apigenin could mediate the occurrence of apoptosis and mediate the occurrence of autophagy by inhibiting the PI3K/Akt/mTOR signaling pathway, while inhibition of autophagy by 3-MA autophagy inhibitor or Atg5 gene silencing could enhance apigenin inhibited hepatoma cell proliferation and apigenin induced hepatoma cell apoptosis, showing that apigenin mediated autophagy could inhibit apoptosis, both of which had a phase antagonistic effect.Melatonin is

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an indoleamine with antioxidant and anti-inflammatory activities.Melatonin has been found to promote the development of autophagy and apoptosis in liver cancer, while inhibition of autophagy enhances melatonin-induced apoptosis when the Beclin-1 gene is silenced or 3-MA autophagy inhibitors are use[31].

Sorafenib has been found to trigger protective autophagy by inhibiting mTOR while triggering mechanisms that promote hepatoma cell death mainly by apoptosis.However, loss of lncRNA SNHG1 could enhance apoptosis and autophagy in sorafenib resistant HCC cells by inhibiting activation of Akt signaling pathway, thereby enhancing the activity of sorafenib[32].Hussain et al.[33]found that sorafenib combined with aescinate over-expressed p62 and LC-II, blocked autophagy and induced apoptosis in advanced HCC cells, and ATG5 knockdown further confirmed the antagonism between autophagy and apoptosis.ASPP2 is an important regulatory molecule for cell survival and death.ASPP2 inhibits autophagy and induces apoptosis through the mTORC1-ERS pathway in TNF-α-induced hepatocellular injury[34].MiR-132 can inhibit the expression of PIK3R3, and LINC00160 can inhibit hepatoma cell apoptosis by competitively binding miR-132 to up-regulate the expression of PIK3R3[35].The above literature shows that autophagy can protect the survival of HCC cells and antagonize the apoptosis of HCC cells.

5. Summary and outlook

In summary, autophagy and apoptosis play an important role in the development of liver cancer, and apoptosis and autophagy interact and are intricate. On the one hand, autophagy-related proteins are involved in the regulation of apoptosis; on the other hand, apoptosis-related proteins are also involved in the regulation of autophagy; in addition, autophagy also has a dual role of lethal autophagy and protective autophagy in liver cancer. Which autophagy is induced depends on multiple factors, such as different drugs, different drug doses, different drug action times, and different stages of the disease. Therefore, exploring the interaction pattern between apoptosis and autophagy and their related regulators is helpful to find effective therapeutic targets and approaches for liver cancer and obtain more satisfactory efficacy.

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