Research Article

Albumin Paclitaxel Compared with 5-Penfluorouracil, Lobaplatin, and Albumin Paclitaxel Combined with 5-Penfluorouracil in the Treatment of Human Gastric Cancer Cell AGS Line Autophagy and Apoptosis

Xingzhen Cheng,1 Fang Yang,2,3 Yang Wang,1 Wei Nie,2 Adarsha Mahendra Upadhyay,1 Maolin Zhang,1 Qian Wang,4 and Zhiqiang Yan4

1Department of Clinical Medicine, Guizhou Medical University, Guiyang 550004, Guizhou, China
2Department of Medical Laboratory Science, Guizhou Medical University, Guiyang 550004, Guizhou, China
3Department of Laboratory Medicine, Affiliated Hospital of Guizhou Medical University, Guiyang 550004, Guizhou, China
4Department of Gastrointestinal Surgery, The Affiliated Hospital of Guizhou Medical University, Guiyang 550004, Guizhou, China

Correspondence should be addressed to Qian Wang; wq5969@sina.com and Zhiqiang Yan; yanzhiqiang@gmc.edu.cn

Received 13 November 2021; Revised 14 April 2022; Accepted 16 April 2022; Published 10 June 2022

Background. Gastric cancer is one of the most common malignant tumors in the world. Albumin paclitaxel (Nab-PTX) is a novel microtubule inhibitor with albumin as the carrier. Several clinical trials are underway in gastric cancer, but the autophagy mechanism of Nab-PTX on gastric cancer is still unclear. The autophagy and apoptosis effects of Nab-PTX compared with 5-pentafluorouracil (5-Fu) and lobaplatin (LBP) in gastric cancer are also unclear. Objective. This article will compare the effects of Nab-PTX, 5-Fu, LBP, and albumin paclitaxel+5-pentafluorouracil (Nab-PTX+5-Fu) on AGS cells from the perspective of autophagy and apoptosis, which is to provide new ideas and experimental evidence for gastric cancer. Method. (1) Experimental groups were control (Ctrl), Nab-PTX, 5-Fu, LBP, and Nab-PTX+5-Fu. (2) CCK-8 assay was used to reflect cell viability and proliferation. (3) Flow cytometry was used to perform the 24-hour apoptosis and cell cycle of each group. (4) Western blot assay was used to investigate autophagy signal proteins LC3I/LC3II, LC3II/LC3I, SQSTM1/p62, Beclin-1, Atg12, Atg5, p-ULK1, p-AMPK, p-mTOR, and apoptosis signal proteins Bax and Bcl-2. Results. Nab-PTX, 5-Fu, LBP, and Nab-PTX+5-Fu inhibited AGS cells’ proliferation and arrested the cell cycle. At the same time, each group increased the apoptosis of AGS cells to various degrees (Nab-PTX+5-Fu > Nab-PTX > 5-Fu > LBP, respectively). The experimental results showed that Nab-PTX and Nab-PTX+5-Fu promoted autophagy and apoptosis of AGS cells. The comparison of Nab-PTX, 5-Fu, and LBP between groups revealed that 5-Fu inhibited autophagy and the expression of apoptosis protein Bax. In LBP, abnormal activation of autophagy downstream, blocking of autophagy flow, abnormal increase of ATG12, and increased expression of apoptosis protein Bax occurred. Further study found that the autophagy upstream mechanism is different. Conclusion. Nab-PTX, 5-Fu, LBP, and Nab-PTX+5-Fu can inhibit cell proliferation, promote cell apoptosis, and induce the difference in autophagy expression. The autophagy difference of this antitumor drug may be related to its inducing apoptosis. Meanwhile, Nab-PTX has a better antitumor effect than 5-Fu and LBP in gastric cancer, and the combination of Nab-PTX+5-Fu has more antitumor advantages.
1. Introduction

According to the Global Cancer Statistics in 2018, gastric cancer has become the fifth most frequently diagnosed cancer in the world (with more than 1,000,000 new cases) and the third leading cause of cancer death worldwide (with an estimated death toll of 783,000) [1]. The burden of gastric cancer is particularly severe in China, where the incidence of gastric cancer accounts for 42.6% of the global incidence and 45% of all gastric cancer-related deaths [2]. Although most early gastric cancer can be cured by endoscopic or surgical resection [3], the prognosis of advanced gastric cancer is still poor, and chemotherapy plays a critical role in advanced gastric cancer. Oral fluorouracil plus oxaliplatin is used as the first-line chemotherapy for unresectable advanced gastric cancer. However, the resistance to classical chemotherapy drugs has become an inevitable problem [5]. Drug resistance has become a significant obstacle to the effective treatment of advanced gastric cancer [6], so it is urgent to develop new treatment ideas and directions for gastric cancer.

At present, among the new chemotherapy drugs in clinical trials for gastric cancer, albumin paclitaxel (Nab-PTX) and lobaplatin (LBP) have broad application prospects. Nab-PTX is a new water-soluble microtubule inhibitor derived from paclitaxel (PTX). Compared with PTX, Nab-PTX does not require special solvents and preuse of steroids to treat tumors [7]. Therefore, Nab-PTX has the potential advantages of fewer side effects and avoiding immunosuppression. At present, a large number of clinical trials of albumin paclitaxel in cancer treatment have shown that albumin paclitaxel has sound antitumor effects [7–10]. The drug resistance of oxaliplatin and cisplatin in cancer treatment cannot be ignored. The LBP, as a third-generation platinum alternative, is characterized by good water solubility, high antitumor activity, and a broad anticancer spectrum. However, it is only approved for the treatment of small cell lung cancer in China at present [11, 12].

2. Materials and Methods

2.1. Main Materials and Instruments. The RPMI 1640, PBS buffer, common pancreatin (containing EDTA), pancreatic without EDTA, and fetal bovine serum were purchased from Biological Industries (BI), and Pierce RIPA buffer was purchased from Thermo. The reagents required by RIPA Lysis buffer were purchased from Shanghai Biyuntian Biotechnology Co., Ltd., and the CCK-8 kit was purchased from Dojindo Laboratories. The Annexin V-FITC/PI, an apoptosis detection kit, was purchased from Becton, Dickinson, and Company, and the cycling kit was also purchased from Becton, Dickinson, and Company. The electrophoresis readers were also purchased from Thermo Scientific.

2.2. Cell Culture. The human gastric cancer cells’ AGS lines were obtained from the Chinese Academy of Sciences Cell Bank. AGS cells were cultured in a complete medium of human gastric cancer cells' AGS lines through the autophagy signaling pathway and compared the differences in autophagy expression and apoptosis of gastric cancer cells induced by Nab-PTX, 5-FU, LBP, and Nab-PTX + 5-Fu.
RPMI-1640 supplemented with 10% fetal bovine serum in humid air containing 5% CO2 and in a constant temperature incubator at 37°C. The cells could be subcultured until 80% growth was achieved.

2.3. Cell Proliferation Assay (CCK-8 Assay). A cell proliferation experiment was carried out using a CCK-8 assay. AGS cells were plated in a 96-well plate at a density of 1 × 10^4 cells/well and were cultured in RPMI 1640 containing 10% FBS. When the cells reach 80%, remove the original culture medium, wash with PBS, and add Nab-PTX (concentration gradient: 0.1 μmol/l, 0.2 μmol/l, 0.4 μmol/l); 5-Fu (concentration gradient: 1 μmol/l, 2 μmol/l, 4 μmol/l); LBP (concentration gradient: 5 μmol/l, 10 μmol/l, 20 μmol/l); Nab-PTX + 5-Fu (concentration gradient: 0.1 μmol/l + 1 μmol/l, 0.2 μmol/l + 2 μmol/l, 0.4 μmol/l + 4 μmol/l); after being cultured for 24 hours, 48 hours, and 72 hours, respectively, 10 μl of CCK-8 solution was added to each well. After incubation at 37°C for 1 hour, measure the absorbance at the wavelength of 450 nm with a microplate reader.

2.4. Flow Cytometry Analysis Assay. First, 2 × 10^5 AGS cells in the logarithmic phase were inoculated into 6-well plates and treated with Nab-PTX (0.2 μmol/l) when they grew to 80%. The, 5-Fu (2 μmol/l), LBP (10 μmol/l), and Nab-PTX + 5-Fu (0.2 μmol/l + 2 μmol/l) were cultured for 24 h, the culture medium was removed, and the cells were washed by precooling with PBS. The cells were lysed with precooling lysis buffer containing 1% protease inhibitor (Thermo), and proteins were collected and denatured. The accumulated proteins were separated by electrophoresis to separate protein molecules of different sizes on the gel and then transferred to the PVDF membrane. Subsequently, a blocking solution was added for sealing. The membrane was incubated with a designated primary antibody. Then, a secondary antibody was added to check the expression of the target protein. Results exposure was performed by ECL chemiluminescence and gel imager. At the same time, the ImageJ software was used for gray value analysis. The above experimental workflow is as follows:

2.5. Protein Extraction and Western Blot Assay. First, 2 × 10^5 AGS cells in the logarithmic phase were inoculated into 6-well plates and treated with Nab-PTX (0.2 μmol/l) when they grew to 80%. The, 5-Fu (2 μmol/l), LBP (10 μmol/l), and Nab-PTX + 5-Fu (0.2 μmol/l + 2 μmol/l) were cultured for 24 h, the culture medium was removed, and the cells were washed by precooling with PBS. The cells were lysed with precooling lysis buffer containing 1% protease inhibitor (Thermo), and proteins were collected and denatured. The accumulated proteins were separated by electrophoresis to separate protein molecules of different sizes on the gel and then transferred to the PVDF membrane. Subsequently, a blocking solution was added for sealing. The membrane was incubated with a designated primary antibody. Then, a secondary antibody was added to check the expression of the target protein. Results exposure was performed by ECL chemiluminescence and gel imager. At the same time, the ImageJ software was used for gray value analysis. The above experimental workflow is as follows:

2.6. Statistical Analysis. SPSS25.0 statistical software was used for analysis. Mean ± standard deviation (X ± S) was used to represent all data, including at least three independent experiments. One-way ANOVA was used for multigroup comparison, and a t-test was used for pair comparison. P < 0.05 was considered statistically significant, P < 0.01 was considered statistically significant, and P < 0.001 was considered statistically significant.
3. Results

3.1. Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu Can Inhibit the Proliferation of AGS Cells. To determine the influence of each group on AGS cell proliferation, we performed CCK-8 cell proliferation detection and analysis. The results showed that compared with the control group (Ctrl), Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu could effectively inhibit the growth of AGS cells. IC50 values of Nab-PTX at 24 h, 48 h, and 72 h are 0.34 μmol/l, 0.095 μmol/l, and 0.081 μmol/l, respectively, as shown in Figure 1(a). For the control test, the IC50 values of single drug 5-Fu and LBP at 24 h, 48 h, and 72 h were 5-Fu, 3.5 μmol/l, 1.5 μmol/l, and 1.0 μmol/l, respectively, and LBP was 19 μmol/l, 5.9 μmol/l, and 4.2 μmol/l, as shown in Figure 1. We can find that the survival rate of each group decreases with the increase of action time, which is time-dependent.

3.2. The Apoptosis Rate of AGS Cells Treated with Nab-PTX + 5-Fu Was Higher Than That of Nab-PTX, 5-Fu, and LBP. To compare the difference in apoptosis among Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu, we performed Annexin V-FITC/PI flow cytometry, and the result showed that the apoptosis rate was 8.00 ± 2.50 in Ctrl (Figure 2(a)); 17.73 ± 1.98 in Nab-PTX (Figure 2(b)); 16.67 ± 2.22 in 5-5-Fu (Figure 2(c)); 13.67 ± 0.40 in LBP (Figure 2(d)); 24.73 ± 4.31 in Nab-PTX + 5-Fu (Figure 2(e)). Compared with the control group, the apoptosis rate of gastric cancer cells induced by each group was as follows: Nab-PTX + 5-Fu > Nab-PTX > 5-Fu > LBP.

The flow cytometry cycle results were analyzed to study the potential mechanism of Nab-PTX inhibiting the growth of gastric cancer cells: the control group was mainly in G1/G0 phase (Figure 3(a)), the Nab-PTX group could arrest the AGS cell cycle in the G2/M phase (Figure 3(b)), and both the 5-Fu and LBP groups could arrest the AGS cell cycle in S phase (Figures 3(c) and 3(d)). Meanwhile, it was observed that although Nab-PTX + 5-Fu could stop the AGS cell cycle in the S phase, the proportion of the S phase increased compared with that of 5-Fu, and the G2/M phase decreased significantly compared with that of NAB-PTX (Figure 3(e)).

3.3. The Autophagy Signals Induced by Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu Were Differentially Expressed. To analyze the difference in autophagy signal expression when Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu acted on AGS cells, we first detected the expression of LC3II/LC3I protein and SQSTM1/p62 by WB, and the results of Western blot autophagy signal protein showed (Figure 4): Nab-PTX and Nab-PTX + 5-Fu. The expression of LC3II/LC3I decreased, and SQSTM1/p62 increased in 5-Fu. The presentation of LC3II/LC3I in LBP decreased, but the absolute values of LC3II and LC3I increased, and the expression of SQSTM1/p62 increased significantly. Then, we detected the expression of Beclin-1 in each group. The presentation of Beclin-1 increased slightly in LBP but decreased in Nab-PTX, 5-Fu, and Nab-PTX + 5-Fu groups. At last, to clarify the changes in the autophagy signal pathway, we detected the expressions of ATG12, ATG5, p-ULK1, p-AMPK, and p-mTOR. The presentation of ATG12 in Nab-PTX, 5-Fu, and Nab-PTX + 5-Fu decreased, while that in LBP increased significantly. ATG5 and p-mTOR decreased slightly in each group. The expressions of p-AMPK and p-ULK1 in 5-Fu and Nab-PTX + 5-Fu increased significantly, while the terms of p-AMPK and p-ULK1 in Nab-PTX increased somewhat, while p-AMPK increased and p-ULK1 decreased significantly in LBP.

3.4. Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu Induced Differential Expression of Apoptosis Signals. To analyze the differences in the expression of apoptosis signal proteins when Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu act on AGS cells, the Western blot assay was used to detect the expression difference of Bax and Bcl-2. The results showed (Figure 5) that compared with the Ctrl group, Bax expression in each group was upregulated, and Bcl-2 in 5-Fu and Nab-PTX + 5-Fu.

4. Discussion

As we all know, Nab-PTX is a new solvent-free 130 nm nano-albumin-binding paclitaxel preparation developed by Celgene, an American biopharmaceutical company. The pharmacological characteristics of albumin paclitaxel are as follows: (1) albumin is reversibly transported through the body by binding to hydrophobic molecules and is quickly released on the cell surface. (2) The drug transmissibility is improved by passing components similar to plasma albumin into interstitial space through endothelial cells [19]. Nab-PTX has been widely used to treat breast cancer, non-small-cell lung cancer, and pancreatic cancer. The Japanese Guidelines for the Treatment of Gastric Cancer approved Nab-PTX to treat advanced gastric cancer in 2013. As a derivative of PTX, Nab-PTX has the same antitumor principle as PTX, which leads to G2/M phase stagnation by promoting tubulin polymerization and stabilizing microtubules, leading to abnormal mitosis of cancer cells and ultimately tumor cell death [20]. In this regard, the CCK8 cell proliferation test was conducted, and it was observed that Nab-PTX at a low dose could effectively inhibit the proliferation of AGS cell lines, and the inhibition rate increased with the increase of concentration and time. Additionally, flow cytometry was used to detect the cell cycle, and we also observed that NAB-PTX could effectively block the AGS cell cycle in the G2/M phase.

In our study, we found that 5-Fu and LBP could also inhibit the proliferation of AGS cell lines. In the cell cycle experiment, we found that both 5-Fu and LBP blocked the cell cycle in the S phase. However, the antitumor mechanisms of 5-Fu and LBP are not all the same. The tool of 5-Fu is to inhibit thymine nucleotide synthase and block the conversion of deoxy pyrimidine nucleotide to thymine nucleotide, thus interfering with DNA synthesis and playing an antitumor role [21, 22]. On the other hand, LBP produces alkylation, destroys DNA molecular structure, and makes cross-linking between bases, resulting in antitumor [23, 24]. Both 5-Fu and LBP ultimately interfere with DNA synthesis, thus arresting the cell cycle in the S phase. Interestingly, we
found that Nab-PTX + 5-FU inhibited the AGS cell cycle in S and G2/M phases while effectively proliferating AGS cells and the proportion of the S phase was increased compared with 5-Fu. In contrast, the G2/M phase was significantly decreased compared with Nab-PTX. In the study of Pascascentilli et al. [25], they found that Nab-PTX combined with 5-FU enhanced the accumulation of cells in the S phase, which was superior to the increase of cells accumulated in the S phase, leading to a sharp decrease of cells in G2 phase. It was speculated that Nab-PTX seemed to interfere with the early stage of the S phase. Further studies are needed to confirm this.

At present, the relationship between autophagy and cancer has always been a hot research topic, and there is no unified conclusion. Autophagy is now thought to play a protective role in cancer development, but autophagy plays the opposite protective role once cancer cells have formed. Autophagy also plays a double role in drug therapy of tumors [15, 26]. Therefore, the role of autophagy in the treatment of AGS cell lines by Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu was further studied. We found that Nab-PTX + 5-Fu and Nab-PTX showed similarity in the downstream of autophagy: LC3II/LC3I expression increased and SQSTM1/P62 expression decreased, while Nab-PTX + 5-Fu expression was highly similar to 5-Fu expression in the upstream of autophagy. The expressions of p-AMPK and p-ULK1 were significantly increased, and the manifestation of Nab-PTX + 5-Fu and 5-Fu in the apoptosis signal was also very similar. The expression of Bcl-2 was significantly increased, while Bax was slightly increased. Studies have shown that excessive activation of autophagy can lead to type II programmed cell death, and excessive activation of

![Figure 1](image-url)
Figure 2: Continued.
Figure 2: Apoptosis in each group of AGS cells. (a) The 24 h apoptosis map of control; (b) the 24 h apoptosis map of Nab-PTX (0.2 μmol/l); (c) the 24 h apoptosis map of 5-F (2 μmol/l); (d) the LBP (10 μmol/l) 24 h apoptosis map; (e) the 24 h apoptosis map of Nab-PTX + 5-F (0.2 μmol/l + 2 μmol/l); (f) apoptotic cells (%) in different groups, analyzed by Annexin V-FITC/PI and using flow cytometry, Q1 = mechanical damage, Q2 = late apoptotic cells, Q3 = live cells, Q4 = early apoptotic cells. All the above experiments were repeated at least three times independently. * P < 0.05, ** P < 0.01. Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu can block the cell cycle at different stages.

Figure 3: Continued.
Figure 3: Continued.
autophagy leads to extreme degradation of mitochondria and other surviving molecules, ultimately leading to apoptosis [27, 28]. Combined with the apoptosis detection results of AGS cells treated by Nab-PTX, 5-FU, and Nab-PTX+5-FU, namely, Nab-PTX + 5-Fu > Nab-PTX > 5-Fu, we speculated that NAB-PTX and NAB-PTX+5-FU might promote the apoptosis of AGS cells through the activation of autophagy. The premise is that autophagy flows smoothly. Beclin-1 is encoded by the BECN1 gene located on human chromosome 17Q21, and Beclin-1 plays a crucial role in regulating autophagic vesicle formation by activating PI3KC3 (also known as Vps34) and promoting the formation of PI3K complex (also known as Vps34 complex) [29]. Currently, it is recognized that BCL-2, an antiapoptotic protein of the Bcl family, is closely related to Beclin-1, which inhibits Beclin-1 activity through the BH3 domain of Beclin-1 [30, 31]. However, Beclin-1 is also regulated by a critical upstream target, ULK1, and AMPK seems to activate complexes involved in autophagy by phosphorylation of Beclin-1 [32]. Although 5-Fu and Nab-PTX + 5-Fu had similar expressions of p-AMPK, p-MTOR, and Bcl-2, the increase of Nab-PTX + 5-Fu was not as obvious as that of 5-Fu, which ultimately led to a significant decrease in Beclin-1 expression of Nab-PTX+5-Fu. This also indicates that the regulation of autophagy is not a simple addition and subtraction relationship but a process of dynamic threshold change. It is worth mentioning that Beclin-1 often appears as a tumor suppressor gene in the process of tumor development. For example, BECN1 single allele deletion is commonly seen in breast cancer, ovarian cancer, and prostate cancer [33]. Beclin-1 expression in other tumors is lower than in normal tissues, but studies have shown that Beclin-1 is overexpressed in gastric cancer tissues. The high expression of Beclin-1 predicts a worse prognosis of gastric cancer [16,33]. Thus, Nab-PTX + 5-Fu treatment of AGS cell lines resulted in a significant decrease in Beclin1, which seems to be a good sign in treating gastric cancer. In addition, further studies are needed to determine the cause for the difference in protein expression between Nab-PTX + 5-Fu, Nab-PTX, and 5-Fu in the upstream and downstream of autophagy, just like looking for a twisted valve between Nab-PTX autophagy and 5-Fu autophagy when the valve is twisted in a particular direction that results in the occurrence of Nab-PTX + 5-Fu autophagy.

Unlike the mechanism of action of Nab-PTX, 5-Fu, and Nab-PTX + 5-Fu, LBP seems to adopt another pathway. Our study found that the expression of LC3II/LC3I decreased in the LBP group, but the absolute value of LC3II and LC3I increased, and the expression of SQSTM1/p62 increased significantly. The presentation of ATG12 increased abnormally, suggesting that the block of autophagy flux might occur in the LBP group [34, 35]. A study on antitumor therapy for lung cancer [36] found that it induced intracellular ROS accumulation by blocking autophagy flux and enhanced the cytotoxicity of cisplatin and PTX to lung cancer cells. The possible mechanism is that hederagenin blocks the autophagy flux, induces the accumulation of mitochondrial ROS, and prevents the damaged mitochondria from being cleared through autophagy, thus enhancing the cytotoxicity of chemotherapy drugs. In addition, components of the molecular mechanism of autophagy also mediate unrelated functions of autophagy [37]. He Liu et al.
Figure 4: The effect of each group on autophagy of AGS cell line. Treatment of gastric cancer cells with Nab-PTX (0.2 μmol/l), 5-Fu (2 μmol/l), LBP (10 μmol/l), and Nab-PTX + 5-Fu (0.2 μmol/l + 2 μmol/l) for 24 hours; after protein extraction, WB experiment was performed. WB results showed the protein expression of LC3II/LC3I, P62, Beclin-1, ATG12, ATG5, p-mULK1, and p-AMPK. All the above experiments were repeated at least three times independently. * P < 0.05, ** P < 0.01.
confirmed that ATG12 has an autophagy-independent role as an oncogene that promotes mitochondrial biogenesis. Due to ATG12 deficiency, mitochondrial biogenesis and cell bioenergetics are reduced, leading to tumor cell death. Swelling of cells and organelles and cytoplasmic bubbles were observed in the cells undergoing distension, which also proved that when ATG12 was deficient, energy deficiency caused damage to the cell ion pump. Coincidentally, Soren Mai et al. [39] found that overexpression of ATG12 leads to improvement of mitochondrial membrane potential, enhancement of ATP production, and antiapoptotic effect. Similar findings were also found in our experiment. LBP and NAB-PTX were identical in the expression of p-AMPK, p-MTOR, Atg5, Bax, Bcl-2, and other proteins. Still, the apoptosis experiment showed that the apoptosis rate of LBP was far lower than that of NAB-PTX, which may be related to the abnormal increase of ATG12 expression caused by LBP. It may also be related to autophagy flow blockage. Mitochondria may play an essential role in LBP-induced drug resistance of AGS cell lines. Notably, the expression level of Beclin-1 in the LBP group was similar to that in normal AGS cells without special treatment, suggesting that the high expression of Beclin-1 in gastric cancer cells does not seem to be beneficial for antitumor therapy.

In our study, differences in p-AMPK and p-ULK1 expressions were observed among the groups, and there was no significant difference in p-MTOR reaching the standard among the groups. Here, we focus on the significance of the differential expressions of p-AMPK and p-ULK1 in each group. First, let us take a look at the structure and function of AMPK: adenosine monophosphoric (AMP) activated protein kinase (AMPK)) is a heterotrimer αβγ complex that competitively binds AMP, ADP, and ATP to three sites in its γ subunit to sense the cellular energy state, of which AMP is the most significant [32, 40]. AMPK promotes autophagy not only directly through phosphorylation of ULK1 but also indirectly through the inactivation of mTORC1 [34]. In addition, AMPK is also a redox sensor and regulator. ROS-induced energy stress can strongly activate AMPK, independent of change in ADP, AMP, and ATP to three sites in its γ subunit to sense the cellular energy state, of which AMP is the most significant [32, 40]. AMPK promotes autophagy not only directly through phosphorylation of ULK1 but also indirectly through the inactivation of mTORC1 [34].

Moreover, AMPK-induced autophagy is a crucial regulator of cell migration [42]. AMPK has been widely recognized as a promising pharmacological target, especially in treating diabetes, obesity, inflammation, and cancer [42, 43].

![Figure 5](image-url)
In short, AMPK’s role in cells is complex. In our study, it was under such complex regulation that the autophagy downstream signals eventually changed significantly. Our experiment observed that LBP and NAB-PTX expressed similar amounts of p-AMPK and p-MTOR but significantly differed in the downstream effects on ULK1. Studies have shown that in addition to AMPK and mTOR regulation, ULK1 is also directly activated by TIP60 [44, 45]. More studies are needed to verify the role of this mechanism in LBP and gastric cancer cells.

Autophagy is involved in cancer growth, prevention, treatment, and drug resistance. At present, research on autophagy and immunotherapy is booming, and KRAS gene mutation leads to increased resistance to epidermal growth factor receptor- (EGFR-) targeted therapy [33]. Porrud et al. studied KRAS wild-type and mutant colorectal cancer cells (CRC) and cancer stem cells (CSCs), showing that 5-Fu can induce CRC apoptotic cell damage but upregulate CSCs autophagy, leading to drug resistance, but inhibition of this autophagy using 5-FU in combination with cetuximab, an anti-EGFR monoclonal antibody, increased the response of cancer stem cells to therapy [46]. As part of signaling pathways such as the RAS/MAPK pathway, BRAF is involved in cell differentiation, cell motility, and apoptosis [33]. In studies of melanoma, BRAF inhibitors can induce autophagy in different ways. BRAF combined with autophagy inhibitors may reverse resistance to BRAF inhibitors [47]. Furthermore, BRAF IncRNA may be another mechanism of tumor proliferation and tyrosine kinase inhibitor (TKI) escape in hepatocellular carcinoma (HCC) [48].

In conclusion, autophagy plays a complex role in treating gastric cancer. Nab-PTX, 5-Fu, LBP, and Nab-PTX + 5-Fu can inhibit cell proliferation, promote cell apoptosis to varying degrees, and induce differences in autophagy expression. The difference in autophagy of this antitumor drug may be related to the apoptosis induced by Nab-PTX. Nab-PTX induces more apoptosis than 5-Fu and LBP, which may be related to the increase in autophagy caused by Nab-PTX. Nab-PTX + 5-Fu induced more apoptosis than Nab-PTX and 5-Fu alone, which may be related to the rise and degree of autophagy induced by Nab-PTX + 5-Fu. In LBP, autophagy-mediated drug resistance may occur. It suggested that Nab-PTX has a better antitumor effect than 5-Fu and LBP in gastric cancer, and the combination of Nab-PTX + 5-Fu has more antitumor advantage. Based on the results of this experiment, for the treatment of gastric cancer, the combination drug still has a huge advantage over the single drug; although the role of autophagy in the antitumor effect of Nab-PTX, 5-Fu, and LBP is different, Nab-PTX is more effective than 5-Fu and LBP. It has autotumor advantages, suggesting that Nab-PTX is feasible for the treatment of gastric cancer and may also increase the efficacy and benefit of gastric cancer patients. At the same time, more studies are required to show the complex relationship between autophagy signal and apoptosis signal and autophagy signal and mitochondrial oxidative stress. In addition, our experiments are only carried out on AGS cells, leading to a certain deviation in our experimental conclusions in gastric cancer, so the findings of this experiment need to be verified on more cell lines.

**Data Availability**

No data were used to support this study.

**Disclosure**

Xingzhen Cheng and Fang Yang are the co-first authors of this article.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Supplementary Materials**

Here, we need to make the following additional remarks. (1) All the above WB results are true and reliable. In the WB Supplementary Materials provided, we attach the original film for explanation. (2) In the FACS experiment, we set the unstained/isotype control to ensure the authenticity of the experiment. Please refer to the FACS Supplementary Instructions for experimental group settings. (3) All data conform to the normal distribution (Gaussian) and allow parametric statistical testing; for this, we attach the original data of the experiments involved (SPSS analysis experiment raw data). (Supplementary Materials)

**References**

[1] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, “Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries,” CA: A Cancer Journal for Clinicians, vol. 68, pp. 394–424, 2018.

[2] F. Wang, L. Shen, J. Li et al., “The Chinese society of clinical oncology (CSCO): clinical guidelines for the diagnosis and treatment of gastric cancer,” Cancer Communications, vol. 39, pp. 01–10, 2019.

[3] J. H. Pyo, H. Lee, B. H. Min et al., “Long-term outcome of endoscopic resection vs. surgery for early gastric cancer: a non-inferiority-matched cohort study,” American Journal of Gastroenterology, vol. 111, pp. 240–249, 2016.

[4] Y. Yamada, K. Higuchi, K. Nishikawa et al., “Phase III study comparing oxaliplatin plus S-1 with cisplatin plus S-1 in chemotherapy-naive patients with advanced gastric cancer,” Annals of Oncology, vol. 26, pp. 141–148, 2015.

[5] C. Holohan, S. Van Schaeybroeck, D. B. Longley, and P. G. Johnston, “Cancer drug resistance: an evolving paradigm,” Nature Reviews Cancer, vol. 13, pp. 714–726, 2013.

[6] B. Yu, D. Gu, X. Zhang, B. Liu, and J. Xie, “The role of GLI2 - ABCG2 signaling axis for 5Fu resistance in gastric cancer,” Journal of Genetics and Genomics = Yi Chuan Xue Bao, vol. 44, pp. 375–383, 2017.

[7] K. Shitara, A. Takashima, K. Fujitani et al., “Nab-paclitaxel versus solvent-based paclitaxel in patients with previously treated advanced gastric cancer (ABSOLUTE): an open-label, randomised, non-inferiority, phase 3 trial,” The Lancet Gastroenterology & Hepatology, vol. 2, pp. 277–287, 2017.
Canadian Journal of Gastroenterology and Hepatology

New England Journal of Medicine, vol. 369, pp. 1691–1703, 2013.

[9] V. Hirsh, A. Ko, R. Pilot, M. F. Renschler, and M. A. Socinski, “Weekly nab-paclitaxel in combination with carboplatin as first-line therapy in patients with advanced non–small-cell lung cancer: analysis of safety and efficacy in patients with diabetes,” Clinical Lung Cancer, vol. 17, pp. 367–374, 2016.

[10] D. Y. Zhang, C. Dmello, L. Chen et al., “Ultrasound-mediated delivery of paclitaxel for glioma: a comparative study of distribution, toxicity, and efficacy of albumin-bound versus cremophor formulations,” Clinical Cancer Research, vol. 26, pp. 477–486, 2020.

[11] S. Dilruba and G. V. Kalayda, “Platinum-based drugs: past, present and future,” Cancer Chemotherapy and Pharmacology, vol. 77, pp. 1103–1124, 2016.

[12] J. Zhou, Y. Kang, L. Chen et al., “The drug-resistance mechanisms of five platinum-based antitumor agents,” Frontiers in Pharmacology, vol. 11, pp. 01–17, 2020.

[13] Y. Zhong, J. Zhang, X. Bai et al., “Lobaplatin in prophylactic hyperthermic intraarterial chemotherapy for advanced gastric cancer: safety and efficacy profiles,” Cancer Management and Research, vol. 12, pp. 5411–5416, 2020.

[14] W. Pei, S. Zhou, J. Zhang et al., “Lobaplatin-based hyperthermic intraarterial chemotherapy for patients with peritoneal metastasis from appendiceal and colorectal cancer: safety and efficacy profiles,” Cancer Management and Research, vol. 12, pp. 12099–12110, 2020.

[15] N. Mizushima and B. Levine, “Autophagy in human diseases,” New England Journal of Medicine, vol. 383, pp. 1564–1576, 2020.

[16] Y. Cao, Y. Luo, J. Zou et al., “Autophagy and its role in gastric cancer,” Clinica Chimica Acta, vol. 489, pp. 10–20, 2019.

[17] O. Camuzard, S. Santucci-Darmanin, G. F. Carle, and V. Pierrefite-Carle, “Autophagy in the crosstalk between tumor and microenvironment,” Cancer Letters, vol. 490, pp. 143–153, 2020.

[18] A. G. Solimando, S. Summa, A. Vacca, and D. Ribatti, “Cancer-associated angiogenesis: the endothelial cell as a checkpoint for immunological patrolling,” Cancer, vol. 12, no. 11, 2020.

[19] N. Desai, V. Trieu, Z. Yao et al., “Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel,” Clinical Cancer Research, vol. 12, pp. 1317–1324, 2006.

[20] V. Sharma and K. Pathak, “Liposoluble system of paclitaxel using modified polysaccharides: in vitro cytotoxicity, apoptosis study, cell cycle analysis, in vitro mitochondrial membrane potential assessment, and pharmacokinetics,” International Journal of Biological Macromolecules, vol. 137, pp. 20–31, 2019.

[21] D. S. Kim, K. Min, and S. K. Lee, “Cell cycle dysregulation is associated with 5-fluorouracil resistance in gastric cancer cells,” Anticancer Research, vol. 40, pp. 3247–3254, 2020.

[22] B. Xie, E. Becker, I. Stuparevic et al., “The anti-cancer drug 5-fluorouracil affects cell cycle regulators and potential regulatory long non-coding RNAs in yeast,” RNA Biology, vol. 16, pp. 727–741, 2019.

[23] S. Hua, X. Kong, B. Chen et al., “Anticancer mechanism of lobaplatin as monotherapy and in combination with paclitaxel in human gastric cancer,” Current Molecular Pharmacology, vol. 11, pp. 316–325, 2018.

[24] J. He and H. Zhang, “The antitumor effect of lobaplatin against Ishikawa endometrial cancer cells in vitro and in vivo,” Biomedicine & Pharmacotherapy, vol. 114, Article ID 108762, 2019.

[25] I. Passacantilli, V. Panzeri, F. Terracciano, G. Delle Fave, C. Sette, and G. Capuroso, “Co-treatment with gemcitabine and nab-paclitaxel exerts additive effects on pancreatic cancer cell death,” Oncology Reports, vol. 39, pp. 1984–1990, 2018.

[26] L. J. Mulcahy and A. Thorburn, “Autophagy in cancer: moving from understanding mechanism to improving therapy responses in patients,” Cell Death & Differentiation, vol. 27, pp. 843–857, 2020.

[27] E. Morselli, L. Galluzzo, O. Kepp et al., “Anti- and pro-tumor functions of autophagy,” Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, vol. 1793, pp. 1524–1532, 2009.

[28] Y. Hsieh, R. Lin, Y. Shen et al., “Flavokawain B and doxorubicin work synergistically to impede the propagation of gastric cancer cells via ROS-mediated apoptosis and autophagy pathways,” Cancers, vol. 12, p. 2475, 2020.

[29] C. He and B. Levine, “The beclin 1 interactome,” Current Opinion in Cell Biology, vol. 22, pp. 140–149, 2010.

[30] D. Glick, S. Barth, and K. F. Macleod, “Autophagy: cellular and molecular mechanisms,” The Journal of Pathology, vol. 221, pp. 3–12, 2010.

[31] D. J. Klionsky, K. Abdelmohsen, A. Abe et al., “Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition),” Autophagy, vol. 12, pp. 1–222, 2016.

[32] A. González, M. N. Hall, S. Lin, and D. G. Hardie, “AMPK and TOR: the Yin and Yang of cellular nutrient sensing and growth control,” Cell Metabolism, vol. 31, pp. 472–492, 2020.

[33] R. M. Usman, F. Razaq, A. Akbar et al., “Role and mechanism of autophagy-regulating factors in tumorigenesis and drug resistance,” Asia-Pacific Journal of Clinical Oncology, vol. 17, pp. 193–208, 2021.

[34] H. Pan, Y. Wang, K. Na et al., “Autophagic flux disruption contributes to Ganoderma lucidum polysaccharide-induced apoptosis in human colorectal cancer cells via MAPK/ERK activation,” Cell Death & Disease, vol. 10, p. 456, 2019.

[35] P. Jiang and N. Mizushima, “LC3- and p62-based biochemical methods for the analysis of autophagy progression in mammalian cells,” Methods, vol. 75, pp. 13–18, 2015.

[36] K. Wang, X. Liu, Q. Liu et al., “Hederagenin potentiated cisplatin- and paclitaxel-mediated cytotoxicity by impairing autophagy in lung cancer cells,” Cell Death & Disease, vol. 11, pp. 01–16, 2020.

[37] L. Galluzzi and D. R. Green, “Autophagy-independent functions of the autophagy machinery,” Cell, vol. 177, pp. 1682–1699, 2019.

[38] H. Liu, Z. He, N. Germić et al., “ATG12 deficiency leads to tumor cell oncosis owing to diminished mitochondrial biogenesis and reduced cellular bioenergetics,” Cell Death & Differentiation, vol. 27, pp. 1965–1980, 2020.

[39] S. Mai, B. Muster, J. Bereiter-Hahn, and M. Jendrach, “Autophagy proteins LC3B, ATG5 and ATG12 participate in quality control after mitochondrial damage and influence lifespan,” Autophagy, vol. 8, pp. 47–62, 2012.

[40] Y. Yan, X. Zhou, H. Xu, and K. Melcher, “Structure and physiological regulation of AMPK,” International Journal of Molecular Sciences, vol. 19, p. 3534, 2018.

[41] S. Wu and M. Zou, “AMPK, mitochondrial function, and cardiovascular disease,” International Journal of Molecular Sciences, vol. 21, p. 4987, 2020.

[42] C. Bressan and A. Saghatelayan, “AMPK-induced autophagy as a key regulator of cell migration,” Autophagy, vol. 17, pp. 828–829, 2021.
[43] D. Garcia and R. J. Shaw, “AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance,” Molecular Cell, vol. 66, pp. 789–800, 2017.
[44] S. Y. Lin, T. Y. Li, Q. Liu et al., “GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy,” Science, vol. 336, pp. 477–481, 2012.
[45] M. Zachari and I. G. Ganley, “The mammalian ULK1 complex and autophagy initiation,” Essays in Biochemistry, vol. 61, pp. 585–596, 2017.
[46] M. Porru, L. Pompili, C. Caruso, A. Biroccio, and C. Leonetti, “Targeting KRAS in metastatic colorectal cancer: current strategies and emerging opportunities,” Journal of Experimental & Clinical Cancer Research, vol. 37, no. 1, p. 57, 2018.
[47] X. Liu, J. Wu, H. Qin, and J. Xu, “The role of autophagy in the resistance to BRAF inhibition in BRAF-mutated melanoma,” Targeted Oncology, vol. 13, no. 4, pp. 437–446, 2018.
[48] A. Gnioni, A. Licchetta, R. Memeo et al., “Role of BRAF in hepatocellular carcinoma: a rationale for future targeted cancer therapies,” Medicina, vol. 55, no. 12, 2019.