Review

DOI: 10.5582/ddt.2023.01013

Drug resistance and new therapies in gallbladder cancer

Yuxin Sun[1,§], Xiaoxuan Li[2,§], Haihong Cheng[1], Shouhua Wang[3], Di Zhou[3], Jun Ding[4,*], Fei Ma[1,5,*]

[1] Department of Oncology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China;
[2] Qingdao University, Qingdao, Shandong, China;
[3] Department of General Surgery, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China;
[4] Department of Biliary and Pancreatic Surgery, Shanghai Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China;
[5] Shanghai Institute for Pediatric Research, Shanghai, China.

SUMMARY Gallbladder cancer (GBC) is a highly aggressive malignancy, which poses significant challenges for timely diagnosis, resulting in a dismal prognosis. Chemotherapy serves as a primary treatment option in cases where surgery is not feasible. However, the emergence of chemoresistance poses a significant challenge to the effectiveness of chemotherapy, ultimately resulting in a poor prognosis. Despite extensive research on mechanisms of chemotherapeutic resistance in oncology, the underlying mechanisms of chemoresistance in GBC remain poorly understood. In this review, we present the findings from the last decade on the molecular mechanisms of chemotherapeutic resistance in GBC. We hope that these insights may provide novel therapeutic and experimental targets for further investigations into this lethal disease.

Keywords Gallbladder cancer, drug resistance, chemotherapy

1. Introduction

Gallbladder cancer (GBC) is a rare malignant neoplasm (*1*). The worldwide incidence of GBC is less than 2/100,000 individuals (*2*). The highest morbidity rates are found in Latin America, the Ganges Valley in Asia, and Poland, with incidence rates among females of 27.3/100,000, 21.5/100,000, and 14/100,000, respectively. The mortality rate of GBC has decreased worldwide but rising in Shanghai, China (*3*). Unfortunately, there has been no significant improvement in the effectiveness of treatment, and the five-year survival rate remains less than 5% (*4*). This is likely due to the difficulty in early detection and diagnosis, as there are no specific clinical symptoms. Moreover, the signs and symptoms that do occur are often masked by other conditions, such as cholecystitis or gallbladder stones. By the time most patients are diagnosed, the condition has progressed to a terminal stage (*5*), and radical surgical treatment, which is the only curative option, is no longer possible. Furthermore, patients who do receive surgery have a higher recurrence rate (*6*). Thus, adjuvant radiotherapy, chemotherapy, and immunotherapy play essential roles in improving the overall survival time of patients with GBC (*7*). Postoperative adjuvant radiotherapy has been shown to be therapeutic in improving patients' survival time (*8*). Chemotherapy is also critical, and the current regimen includes gemcitabine (GEM), 5-fluorouracil (5-FU) combined with cisplatin (DDP), and oxaliplatin (*7*). Recent advances in genome sequencing technology have led to the identification of potential genetic aberrations in patients with GBC, highlighting the increasing importance of targeted therapy in GBC treatment.

Given that only a small proportion of GBC patients are eligible for surgery, chemotherapy is typically the primary option for patients with unresectable or metastatic disease. GEM has demonstrated efficacy as a chemotherapy drug for GBC (*9*), with reported tumor response rates ranging from 10% to 30% and a median survival time of 8.1 months. However, GBC cells exhibit significant resistance to this drug and present a significant challenge for effective treatment. What's more, rapid drug resistance development has also become a bottleneck for other chemotherapy drugs like DDP. What's worse, the emergence of multidrug resistance (MDR) is particularly concerning (*10*), as it causes over 90% of deaths in cancer patients treated with traditional or novel chemotherapeutic drugs (*11*). Hence, research efforts must focus on overcoming drug resistance in GBC.

Tanweer Haider *et al.* provides a precise summary

of the mechanisms of chemotherapy resistance in tumors (*12*). The resistance was classified into intrinsic and extrinsic categories based on the involved factors. Intrinsic resistance refers to those specific elements present in the cancer cell or tissue itself before chemotherapy, which can reduce the effectiveness of chemotherapy drugs for particular cancers. Extrinsic drug resistance, also known as acquired drug resistance, is a complex mechanism involving the acquisition of gene mutations and activation of MDR-related signaling pathways. The mechanisms of drug resistance include various factors such as tumor microenvironmental (TME) factors, tumor heterogeneity, drug inactivation, reduced drug influx, increased drug outflow, changes in DNA repair and epigenetic effects, inhibition of apoptotic pathways and autophagy, epithelial-mesenchymal transformation (EMT), and changes in membrane lipids. The main causes of MDR include overexpression of ATP-binding cassette (ABC) superfamily membrane transporters such as p-glycoproteins (P-GP) and MDR-related proteins, inactivation of pathways associated with apoptosis inhibition, and enhancement of DNA self-repair ability. This review examines the mechanisms of chemotherapy resistance and summarizes the latest research advances in overcoming chemotherapy resistance in GBC over the past decade.

2. Research progress on the mechanism of chemotherapeutic resistance in GBC

2.1. TME

TME, consisting of multiple cell types (*e.g.*, endothelial cells, fibroblasts, immune cells) and extracellular components (*e.g.*, cytokines, growth factors, hormones, extracellular matrix), is a critical factor that affects the efficacy of chemotherapy (*13*). Changes in the TME could contribute to the development of drug resistance. Vascular endothelial growth factors (VEGFs) and fibroblast growth factors (FGFs) are key factors in TME that promote tumor growth and survival and have been implicated in chemotherapy resistance in cancer (*14*).

Leptin is an adipose-tissue-derived hormone that is involved in regulating glycometabolism, lipometabolism, and energy metabolism (*15*). Through the signal transducer and activator of transcription 3 (STAT3)/CCAAT/enhancer binding protein delta (CEBPD)/myeloid cell leukemia-1 (MCL1) axis, leptin has been found to induce mitochondrial fusion and inhibit GBC cell apoptosis induced by gemcitabine (*16*). In this signaling pathway, STAT3 acts as a leptin-regulated transcriptional activator, while CEBPD, a transcription factor of the C/EBP family, is inactivated in various cancers (*17*). *MCL1*, a target gene of CEBPD that responds to leptin, is overexpressed in various tumors (*18*). Research has shown that leptin can induce the phosphorylation of STAT3 in GBC cells, leading to increased expression of CEBPD and promoting the transcription of the MCL1 gene (*16*). This, in turn, enhances the anti-apoptotic ability and chemotherapy resistance of GBC cells by enabling mitochondrial fusion and promoting mitochondrial function. Therefore, increasing leptin secretion and promoting the CEBPD/MCL1 axis may enhance chemotherapy resistance in GBC (Figure 1). This study sheds light on the underlying mechanisms contributing to the poor prognosis of obese patients.

2.2. Anti-apoptosis effects

Apoptosis is a form of programmed cell death that plays a critical role in chemotherapy response (*19*). Resistance to chemotherapy can result from various factors that affect the expression of apoptotic cytokines (*11*). The caspase family and the B-cell lymphoma-2 (Bcl-2) family are the two factors typically involved in apoptosis. The caspase family is regarded as the primary mediator of apoptotic cell death, while the Bcl-2 family serves as a crucial regulatory component of the intracellular apoptotic pathway with varying effects on apoptosis (*20*). Bax, one of the pro-apoptotic Bcl-2 proteins, is a negative regulator of cell survival, while Bcl-2 proteins, also in the Bcl-2 family, play an inhibitory role in apoptosis. Numerous factors can affect apoptosis by regulating the expression of these molecules. For instance, estrogen can stimulate the expression of BCL-2, thereby promoting drug resistance in breast cancer (*21*).

Olfactomedin-4 (OLFM4) is a glycoprotein belonging to the OLFM family and is known to exhibit anti-apoptotic properties (*22,23*). Studies have revealed that OLFM4 expression is significantly increased during the progression of chronic cholecystitis to atypical hyperplasia and ultimately to GBC (*24*). Furthermore, genetic knockout of OLFM4 has been shown to result in a marked decrease in tumor proliferation and invasion (*25*). Additionally, cells cultured in the absence of

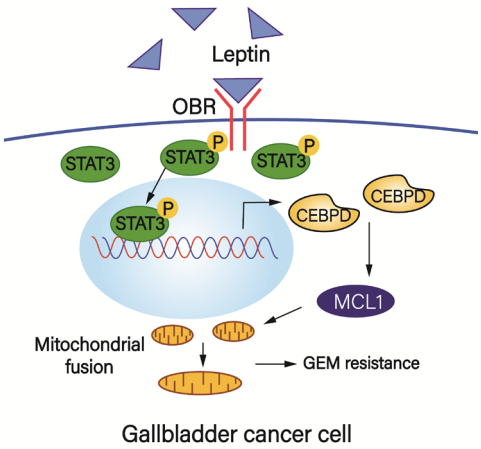

Figure 1. High expression of leptin promotes chemoresistance of GBC cells to GEM by activating the STAT3/CEBPD/MCL1 axis.

OLFM4 have been observed to exhibit reduced levels of ADP-ribosylation factor-like 6 interacting protein 1 (ARL6IP1), another anti-apoptotic cytokine. This implies that the OLFM4-ARL6IP1-caspase 3 axis plays a significant role in regulating chemotherapy resistance. Notably, the downregulation of OLFM4 has been found to increase the expression of caspase 3 and sensitize cells to chemotherapy with DDP, both *in vitro* and *in vivo*. Calreticulin (CRT), located in the endoplasmic reticulum, is another factor that activates anti-apoptotic effects (*26*). CRT is involved in various cellular metabolic processes, including calcium handling, cell adhesion, and migration (*27*). Upregulation of CRT has been observed in several cancers, including lung cancer, pancreatic cancer, and GBC, and it positively correlates with tumor size. Inhibition of CRT has been shown to induce apoptosis and suppress the activation of p-Akt, which enhances the anti-cancer efficacy of GEM (*28*). The PI3K/Akt pathway has previously been reported to increase cancer cell chemoresistance (*29*). Therefore, inhibiting OLFM4 and CRT may increase chemotherapeutic sensitivity and improve treatment efficacy.

MicroRNAs (miRNAs) are small, 20-22 nucleotide molecules that act as post-transcriptional regulators, playing a significant role in cancer development, including GBC (*30*). Among them, miR-125b-5p is a down-regulated miRNA in drug-resistant GBC cells. Recent research has revealed that miR-125b-5p down-regulates the expression of Bcl2, promoting apoptosis and enhancing the chemotherapy sensitivity of GBC cells to DDP (*31*). Hence, the downregulation of miR-125b-5p expression in GBC cells leads to chemotherapy resistance.

MiR-205-5p is another type of miRNA with potential as a diagnostic biomarker of cancer, capable of reducing tumor chemoresistance. Peripheral blood samples from GBC patients show reduced expression of miR-205-5p. Protein kinase C (PRKCE) is associated with tumor invasiveness and is upregulated in various cancers, including GBC. MiR-205-5p inhibits the expression of PRKCE, and its overexpression can promote GEM-induced apoptosis by increasing the sensitivity of GBC cells to GEM (*32*). Furthermore, increased expression of miR-205-5p and silencing of PRKCE can promote the expression of Bax and activate caspase 3. Therefore, the decreased expression of miR-205-5p leads to increased chemotherapy resistance in GBC cells (*33,34*).

MiR-31 is a miRNA that is down-regulated in DDP-resistant GBC cells. *Src* is a proto-oncogene (*35*) and a direct target of miR-31, whose expression is inhibited by miR-31. Akt, as one of the downstream kinases of Src, is implicated in the anti-apoptotic effect by disrupting the balance of Bcl-2 family proteins (*36*). The downregulation of miR-31 expression promotes the expression of activated p-Src and further increases the level of activated p-Akt. Ultimately, a decrease in Bax and an increase in Bcl-2 expression attenuate DDP-induced apoptosis (*37*). Therefore, the reduced expression of miR-31 leads to increased resistance to chemotherapy in GBC cells.

The acquisition of drug resistance in GBC is closely associated with anti-apoptotic effects (Figure 2). Studies investigating the mechanisms of apoptotic resistance have predominantly focused on traditional apoptotic pathways, such as Bax/Caspase3. However, these studies typically only describe a linear relationship between a gene and a downstream target protein. Apoptotic pathways are interconnected and form a complex network of structures. Thus, it is imperative that researchers integrate their studied genes into this intricate network of apoptotic pathways in order to more systematically explain how a series of linkage changes culminate in the development of apoptosis resistance in gallbladder cancer cells. Furthermore, imbalances in microRNA expression frequently arise during GBC development, which can influence cancer cell phenotype and elucidate the mechanism behind drug resistance. However, reversing these expression suppressions *in vivo* remains a significant challenge.

2.3. Autophagy effect

Recent studies have demonstrated that autophagy plays a dual role in tumor chemotherapy resistance and sensitization. Moderate autophagy can increase resistance to chemotherapeutic drugs, while excessive autophagy can prevent the formation of drug resistance, leading to accelerated cell death (*38*). Many factors are involved in cellular autophagy, including recombinant phosphoglycerate kinase 1 (PGK1), a critical enzyme that catalyzes glycolysis, and its activity has been linked to autophagy activation (*39*).

Long non-coding RNAs (lncRNAs) are a class of transcripts that are longer than 200 bases and lack protein-coding potential. These transcripts play vital roles in multiple biological processes of cancer progression, such as proliferation, apoptosis, epithelial-mesenchymal transition, and autophagy (*40*). Among the upregulated lncRNAs, lncRNA ENST00000425894, termed gallbladder cancer drug resistance-associated lncRNA1 (GBCDRlnc1), participates in the chemoresistance of gallbladder cancer cells (*41*). GBCDRlnc1 expression is increased in GBC tissues and can interact directly with PGK1, upregulating its protein level by inhibiting PGK1 ubiquitination in GBC cells *in vitro*, thereby inducing autophagy-related chemical resistance in GBC cells (*41*).

Although the mechanisms of autophagy are rarely studied in GBC, cellular autophagy plays a vital role in the development of drug resistance. For instance, GEM can cause protective autophagy by inhibiting the Akt/mTOR signaling pathway in various tumors, including GBC, resulting in tumor chemotherapy resistance (*42*). Moreover, enhanced autophagy activity in Doxorubicin (DOX)-resistant gallbladder carcinoma cells promotes

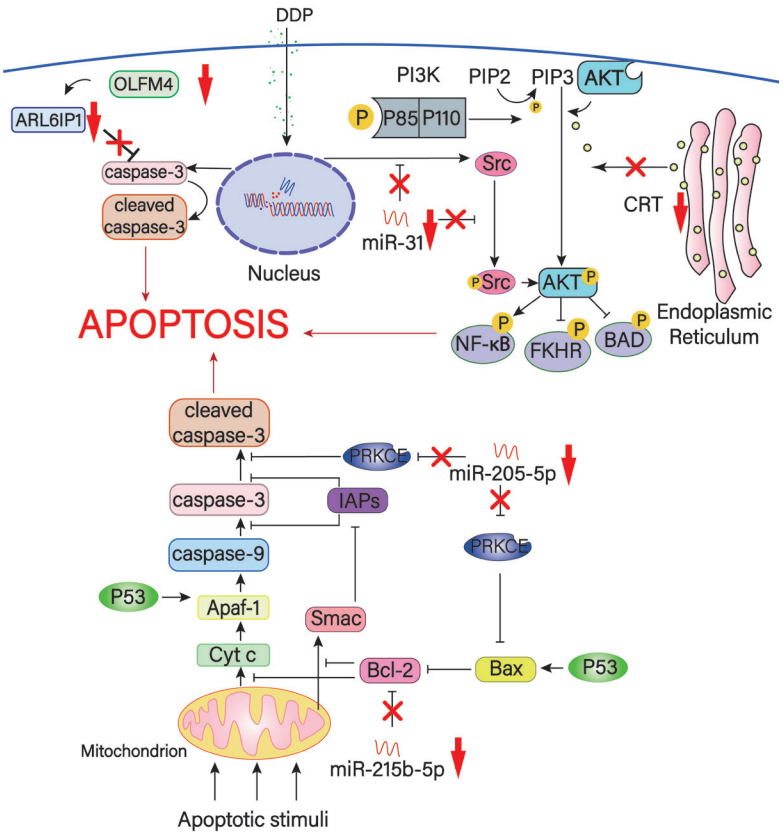

Figure 2. Molecular pathway diagram associated with apoptosis. (*i*) OLFM4 exerts its effect on cell apoptosis through the OLFM4-ARL6IP1-caspase 3 axis. (*ii*) CRT influences the apoptosis through the PI3K/AKT pathway. (*iii*) miR-125b-5p promotes cell apoptosis by affecting the expression of Bcl-2. (*iv*) miR-205-5p affects cell apoptosis through the PRKCE/Bax/caspase 3 pathway. (*v*) miR-31 influences cell apoptosis through the Src/Akt pathway.

drug resistance. Therefore, further research on cellular autophagy is necessary to develop strategies to combat drug resistance in GBC.

2.4. Drug efflux

The overexpression of membrane efflux pumps, such as P-GP, plays a crucial role in MDR, primarily through increased drug efflux (*43*). P-GP, a transmembrane transporter encoded by the multi-drug resistance gene 1 (MDR1), uses ATP-dependent mechanisms to transport drugs out of cells, thereby conferring resistance to cancer cells (*44*).

One miRNA that is significantly downregulated in GBC is miR-218-5p. It inhibits the activation of MDR1/P-GP by downregulating the expression of PRKCE. Therefore, decreased expression of miR-218-5p relieves the inhibitory effect on PRKCE, resulting in increased levels of MDR1/P-GP and drug outflow, ultimately promoting resistance of GBC cells to GEM and DDP.

Another downregulated miRNA in drug-resistant GBC cells is miR-145. MRP1, a critical component in developing chemoresistance, is a target of miR-145 (*45*). MiR-145 reduces the expression level of MDR1/P-GP by promoting the direct degradation of MRP1 mRNA, leading to increased drug efflux (*46*). Consequently, decreased expression of miR-145 leads to increased levels of MDR1/P-GP and enhanced resistance of GBC cells to GEM and DDP. Therefore, miRNAs play multiple roles in the development of gallbladder cancer, and it is important to investigate and reveal the functions of additional miRNAs in the mechanisms underlying GBC.

The deregulation of the mammalian target of rapamycin (mTOR), an atypical serine/threonine kinase, is implicated in the pathogenesis of GBC. mTOR plays a critical role in regulating cell proliferation, growth, differentiation, migration, and survival by modulating numerous cellular signaling pathways (*47*). Inhibition of the mTOR signaling pathway has been shown to suppress the synthesis of MDR1 induced by 5-FU and ultimately promote the chemosensitivity of GBC cells to 5-FU (*48*).

NADPH oxidase 1 (NOX1), a membrane-bound enzyme that is up-regulated in GBC, is a significant source of reactive oxygen species (ROS). Hypoxia-inducible factor 1a (HIF1a) is a master regulator of cellular responses to ROS and plays an essential role in tumorigenesis (*49*). Studies have demonstrated that increased expression of NOX1 can enhance intracellular ROS levels, which then activate the HIF-1A/MDR1 pathway. This leads to increased drug efflux and cisplatin

resistance in GBC cells (*50*).

This suggests that overexpression of P-GP plays a pivotal role in the acquisition of multidrug resistance in GBC (Figure 3). However, the regulation of P-GP expression is a complex and multifaceted process. Thus, developing a single agent that can effectively downregulate P-GP expression *in vivo* presents a significant challenge. Nonetheless, such an agent would be valuable in enhancing the efficacy of multiple chemotherapeutic agents used in combination therapy for GBC.

2.5. DNA repair alteration (DDR)

DDR is a critical mechanism for repairing direct or indirect DNA damage induced by chemotherapy. DDR can be activated to restore the dysfunctional pathway, thus increasing DNA repair activity and drug resistance (*51*). The robustness of the intracellular DDR is a critical determinant of chemotherapy sensitivity.

Long non-coding RNA myosin light chain kinase antisense RNA 1 (MYLK-AS1), which is upregulated in GBC (*52*), acts as a regulator in this process. Its target is the polycomb group (PcG) family member, zeste homologous enhancer 2 (EZH2). EZH2 is a critical regulator of cell cycle progression, autophagy, and apoptosis, promoting DNA repair and inhibiting cell senescence, thereby increasing the DNA repair capacity of cancer cells (*53*). Thus, overexpression of MYLK-AS1 targets miR-217, which increases the expression of EZH2, leading to increased DNA repair in GBC cells and ultimately promoting resistance to chemotherapy drugs.

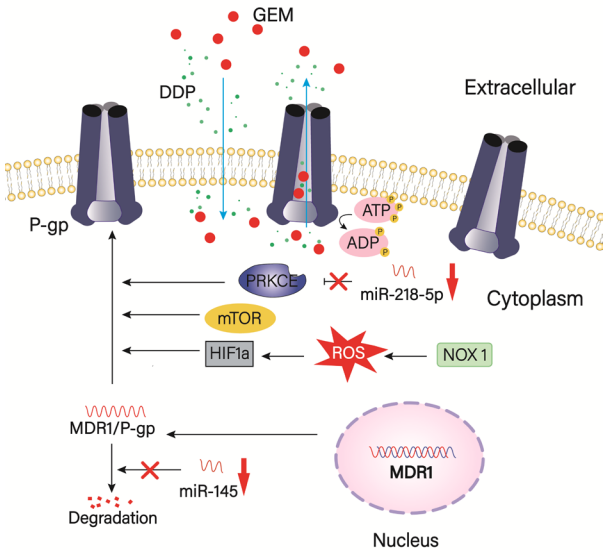

Figure 3. Diagram of cellular efflux mechanisms. (*i*) miR-218-5p inhibits the activation of MDR1/P-GP by downregulating the expression of PRKCE. (*ii*) miR-145 reduces the expression levels of MDR1/P-GP by directly degrading MRP1 mRNA. (*iii*) mTOR inhibits the synthesis of MDR1 induced by 5-FU. (*iv*) NOX1 increases intracellular ROS levels, thereby activating the HIF-1A/MDR1 pathway.

2.6. EMT

The EMT is a physiological process in which epithelial cells undergo transdifferentiation to form motile mesenchymal cells, playing an essential role in wound healing and cancer progression (*54*). Methyl-CpG binding domain protein 1 (MBD1), a transcriptional repressor that mediates DNA methylation, has also been implicated in tumor development and progression (*55*). Downregulation of MBD1 expression significantly increased the Gem sensitivity of gallbladder carcinoma cells (*56*). Furthermore, E-cadherin expression, an epithelial marker, was increased upon MBD1 knockdown, suggesting that MBD1 may promote EMT in GBC cells by suppressing e-cadherin expression. Hence, MBD1-mediated EMT may contribute to the development of chemotherapy resistance in GBC.

In conclusion, research on drug resistance mechanisms in gallbladder cancer was limited until 2010 due to the rarity of the disease and low diagnosis rates. However, with increased health awareness and clinical diagnoses, more patients with GBC receive chemotherapy, drawing attention to chemotherapy resistance in this malignancy. Among the various mechanisms studied, apoptosis and exocytosis are the primary focuses of research, providing insights for future clinical treatments. Additionally, investigating changes in the tumor microenvironment, cellular autophagy, and DNA repair mechanisms could enhance our understanding of chemotherapy resistance in GBC and lead to more effective treatment strategies.

3. Study of new agents and drugs against drug resistance

3.1. Agents at the basic experimental stage

Bufalin, a cardiotonic steroid (CTS) derived from traditional Chinese medicine toad venom, has been shown to exhibit multiple pharmacological activities including anti-tumor effects (*57*). One study demonstrated that bufalin can effectively downregulate Mcl-1, an important anti-apoptotic protein of the Bcl-2 protein family that helps cancer cells evade drug attacks. This downregulation leads to increased apoptosis of GBC cells and improved sensitivity of gallbladder cancer cells to chemotherapy drugs (*58*). Additionally, bufalin can suppress the proliferation and metastasis of tumor cells by impeding the MEK/ERK signaling pathway, reduce self-renewal and drug resistance of tumor stem cells by inhibiting the PI3K/Akt pathway, thus making it a promising candidate for treating patients with GBC resistant to conventional chemotherapy.

Maslinic acid (MA), a pentacyclic triterpene acid, has been shown to inhibit nuclear factor-κB (NF-κB) survival signaling pathways (*59*). By inhibiting NF-κB, MA is able to modulate the expression of regulatory factors for

cell proliferation (cyclin D1), apoptosis (Bax and Bcl-2), and metastasis (MMP-2 and MMP-9). Compared to treatment with GEM alone, the combination of MA and GEM resulted in significant downregulation of Cyclin D1 and Bcl-2 expression, while Bax expression was significantly increased, indicating that MA can inhibit cell proliferation, promote apoptosis, and enhance the sensitivity of GBC cells to chemotherapy (*60*).

Hispidulin, a flavonoid naturally occurring in the traditional Chinese medicinal herb *Salvia involucrate*, has been shown to increase caspase-3 activity and decrease BCL-2 expression while increasing Bax expression in GBC cells. Moreover, hispidulin can inhibit the HIF-1A/MDR1 pathway *via* AMPK signaling, resulting in decreased expression of P-GP and promoting the chemotherapy sensitivity of GBC cells (*61*).

Chloroquine, an autophagy inhibitor, has been found to enhance the induction of apoptosis and cell cycle arrest of GBC cells *in vitro* when used in combination with GEM. Furthermore, chloroquine can reduce the resistance of GBC cells to GEM by inhibiting autophagy and has also demonstrated enhanced tumor inhibition *in vivo* (*62*).

Cordycepin, a bioactive compound found in species of the genus *Cordyceps*, has shown promising results in promoting the sensitivity of GBC cells to chemotherapy drugs such as GEM and 5-FU by inhibiting the activation of mTORC1 and down-regulating the expression of MDR/HIF-1α *via* the AMPK signaling pathway (*63*).

Verapamil, a classical chemical sensitizer, has been shown to inhibit the transport function of P-GP by inhibiting the expression of MRP1. When combined with platinum-based therapy, cryotherapy with verapamil significantly increases the chemical sensitivity of GBC cells (*64*).

Tamoxifen is a commonly used anti-tumor drug with various beneficial effects. Research has demonstrated that tamoxifen can enhance the inhibition of cell activity and apoptosis induced by DDP (*65*). One crucial factor that determines GBC chemical resistance is the intracellular ROS level. Tamoxifen has been shown to induce ROS production and promote GBC apoptosis by inactivating the Nrf2 signal and increasing the expression of CYP.

Osi-027 is a novel ATP competitive inhibitor of mTORC1 and mTORC2 that can effectively inhibit the synthesis of MDR1, down-regulate the expression of MDR1 induced by 5-FU, and enhance the sensitivity of GBC cells to 5-Fu (*48*).

In general, most of these new agents are drugs that have shown antitumor effects in other cancer studies. The research results are only the antitumor phenomena observed in basic experiments and the superficial exploration of the anti-tumor mechanism. Few are used in clinical trials. What's more, the appropriate concentration, specificity, and toxicity of these small molecule drugs to normal tissues remain unknown. Only few agents are actually developed to combat resistance or have the significance to provide guidance for clinical drug use. The ultimate goal of scientific research is to use it to cure patients and benefit people's health. Therefore, there is a need to accelerate the pace of new drug development research.

3.2. The improvement of traditional chemotherapy drugs

NUC-1031 is a promising chemotherapy drug for the treatment of GBC, which has been developed through the application of ProTide technology to transform GEM into a phosphoramidate (*66*). Like GEM, NUC-1031's cytotoxic effect on cancer cells is largely attributed to the generation of the triphosphate form of the nucleotide analog (di-fluoro-deoxycytidine triphosphate [dFdCTP]). However, NUC-1031 can generate and maintain higher concentrations of dFdCTP inside the tumor cell than GEM due to its unique properties. Firstly, the phosphoramidate moiety enables NUC-1031 to enter the cancer cell, independent of the presence of nucleoside transporters. Secondly, once NUC-1031 has entered the cell, the protective group is cleaved off and releases an activated, monophosphorylated form of GEM (dFdCMP). This delivery of dFdCMP obviates the need for the activating enzyme, deoxycytidine kinase, which drives the rate-limiting phosphorylation of gemcitabine. dFdCMP is rapidly converted to di-fluoro-deoxycytidine diphosphate and then the key anticancer metabolite, dFdCTP. Thirdly, NUC-1031 is not subject to breakdown by cytidine deaminase (CDA), which is a key resistance mechanism in GBC. Due to its ability to overcome all three key resistance mechanisms, NUC-1031 achieves much higher levels of the active anticancer metabolite, dFdCTP, than GEM. This mechanism of action has been illustrated in previous publications on NUC-1031 (*67*). Furthermore, the agent has demonstrated safety and tolerability in phase 1 clinical trials and is currently being prepared for later clinical trials.

Capecitabine is an oral prodrug of 5-FU, which exerts its cytotoxic effects by inhibiting DNA synthesis, RNA processing, and protein synthesis. S-1 is another oral anticancer drug that consists of a combination of tegafur (a 5-FU prodrug), gimeracil, and oteracil potassium. Gimeracil acts as an inhibitor of dihydropyrimidine dehydrogenase, thereby increasing the concentration of fluorouracil in the blood and tumor tissue. Oteracil potassium reduces gastrointestinal toxicity by suppressing the phosphorylation of fluorouracil in the gastrointestinal tract. The BILCAP (compare capecitabine with observation following resection of biliary tract cancer) phase 3 trial conducted in the UK in 2019 demonstrated that capecitabine can improve overall survival after resection of biliary tract cancer when used as adjuvant chemotherapy following surgery (*68*). A phase 3 clinical trial (JCOG1202, ASCOT) was conducted in Japan in 2023, which compared adjuvant S-1 with observation in

resected biliary tract cancer. This study showed that S-1 as adjuvant therapy had a comparable survival effect to capecitabine but with fewer side effects for GBC (*69*).

These improvements to traditional chemotherapeutics are expected to be used in clinical applications and bring benefits to patients soon.

3.3. Molecular targeted therapy

Molecular targeted therapy represents a critical adjunct to conventional chemotherapy for advanced, chemotherapy-resistant GBC. Regorafenib is a multikinase inhibitor that targets several pathways, including angiogenesis *via* vascular endothelial growth factor receptors 1-3 and TIE2, oncogenesis by inhibiting the downstream pathways of KIT, RET, RAF1, and BRAF, modulation of the tumor microenvironment by blocking the activity of intracellular domains of platelet-derived growth factor receptor and fibroblast growth factor receptor, and activation of tumor immunity through colony-stimulating factor 1 receptor. In phase II trials (*70-72*), regorafenib was found to be active and significantly increased the median progression-free survival in patients with locally advanced/metastatic GBC that progressed after GEM/DDP-based chemotherapy. Additionally, other agents such as varlitinib, trastuzumab, surufatinib, lenvatinib, and others have also undergone phase 2 trials and demonstrated promising results in prolonging the survival time of GBC patients.

4. Conclusion and prospects

Patients diagnosed with GBC often miss the opportunity for surgery due to late detection, and thus chemotherapy becomes a crucial treatment option to prolong patient survival. The initial use of commonly used clinical chemotherapeutic agents such as gemcitabine and cisplatin has shown promising therapeutic effects. However, the development of chemoresistance has diminished the effectiveness of these agents and hindered their ability to provide long-term support for patients. Therefore, addressing chemotherapy resistance has become an important issue in extending patients' lives.

In the past decade, drug resistance in GBC has gained unprecedented attention, and researchers are addressing this challenge on two fronts. Firstly, efforts have focused on understanding the mechanisms underlying resistance. Alterations in apoptotic pathways and increased extracellular derivation have been identified as key contributors to drug resistance, while changes in miRNA expression seem to play a crucial role in the development of resistance. Secondly, researchers are investigating the development of new drugs that can overcome resistance in GBC cells, which arise when conventional chemotherapeutic agents are used.

To date, significant efforts have been dedicated to eliminating drug resistance as a therapeutic strategy and improving its efficacy. While studies have reported findings on various mechanisms that contribute to the development of drug resistance, there are limited in-depth and continuous studies available to systematically explain the mechanism of drug resistance in gallbladder cancer. Additionally, validation in large multicenter clinical trials is still challenging as gallbladder cancer is relatively rare and the number of patients with the disease per hospital is small. With regards to new small molecule drugs, although they have shown remarkable inhibitory effects on tumor cells in basic experiments, it is still a long road ahead before they can be clinically tested to benefit tumor patients. As such, further research and development are necessary to improve the understanding and management of drug resistance in gallbladder cancer.

In the future, further attention should be given to the following aspects: In basic research, emphasis should be placed on the mechanisms of drug efflux and apoptosis, with a particular focus on changes in various pathways during drug resistance, and systematic studies should be conducted to identify the key points of drug resistance. At present, correlation studies on MDR are too lacking to form a systematic study. Since the generation of MDRs is responsible for almost 90 percent, it should be the most important point for future basic research. The elucidations of the mechanisms and pathways of MDR can lead to the design of new drugs, targeted drugs and the most efficient way to benefit patients.

Funding: This work was supported by the National Natural Science Foundation of China (grant No.81802337), Shanghai Jiao Tong University (grant No.YG2017MS74), and the Shanghai Health Committee (grant No. 2020404447).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Misra S, Chaturvedi A, Misra NC, Sharma ID. Carcinoma of the gallbladder. Lancet Oncol. 2003; 4:167-176.
2. Sharma A, Sharma KL, Gupta A, Yadav A, Kumar A. Gallbladder cancer epidemiology, pathogenesis and molecular genetics: Recent update. World J Gastroenterol. 2017; 23:3978-3998.
3. Hsing AW, Bai Y, Andreotti G, Rashid A, Deng J, Chen J, Goldstein AM, Han TQ, Shen MC, Fraumeni JF, Jr., Gao YT. Family history of gallstones and the risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China. Int J Cancer. 2007; 121:832-838.
4. Goetze TO. Gallbladder carcinoma: Prognostic factors and therapeutic options. World J Gastroenterol. 2015; 21:12211-12217.
5. Hong D. Guidelines for diagnosis and treatment of gallbladder cancer. Journal of hepatopancreatobiliary Surgery. 2020; 32:664-666.
6. Patkar S, Ostwal V, Ramaswamy A, Engineer R, Chopra S, Shetty N, Dusane R, Shrikhande SV, Goel M. Emerging

role of multimodality treatment in gall bladder cancer: Outcomes following 510 consecutive resections in a tertiary referral center. J Surg Oncol. 2018; 117:372-379.
7. Zheng CJ, Zou H, Zhang XW. Progress in the comprehensive treatment of gallbladder carcinoma. J Mod Oncol. 2019; 27:2954-2957.
8. Mantripragada KC, Hamid F, Shafqat H, Olszewski AJ. Adjuvant therapy for resected gallbladder cancer: Analysis of the National Cancer Database. J Natl Cancer Inst. 2016; 109:djw202.
9. Nakamura M, Nakashima H, Abe T, Ensako T, Yoshida K, Hino K. Gemcitabine-based adjuvant chemotherapy for patients with advanced gallbladder cancer. Anticancer Res. 2014; 34:3125-3129.
10. Ding P, Gao Y, Wang J, Xiang H, Zhang C, Wang L, Ji G, Wu T. Progress and challenges of multidrug resistance proteins in diseases. Am J Cancer Res. 2022; 12:4483-4501.
11. Bukowski K, Kciuk M, Kontek R. Mechanisms of multidrug resistance in cancer chemotherapy. Int J Mol Sci. 2020; 21:3233.
12. Haider T, Pandey V, Banjare N, Gupta PN, Soni V. Drug resistance in cancer: Mechanisms and tackling strategies. Pharmacol Rep. 2020; 72:1125-1151.
13. Sethi T, Rintoul RC, Moore SM, MacKinnon AC, Salter D, Choo C, Chilvers ER, Dransfield I, Donnelly SC, Strieter R, Haslett C. Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: a mechanism for small cell lung cancer growth and drug resistance *in vivo*. Nat Med. 1999; 5:662-668.
14. Sun Y. Tumor microenvironment and cancer therapy resistance. Cancer Lett. 2016; 380:205-215.
15. Zhang Y, Chua S, Jr. Leptin function and regulation. Compr Physiol. 2017; 8:351-369.
16. Wang WJ, Lai HY, Zhang F, Shen WJ, Chu PY, Liang HY, Liu YB, Wang JM. MCL1 participates in leptin-promoted mitochondrial fusion and contributes to drug resistance in gallbladder cancer. JCI Insight. 2021; 6:e135438.
17. Lai HY, Hsu LW, Tsai HH, Lo YC, Yang SH, Liu PY, Wang JM. CCAAT/enhancer-binding protein delta promotes intracellular lipid accumulation in M1 macrophages of vascular lesions. Cardiovasc Res. 2017; 113:1376-1388.
18. Brotin E, Meryet-Figuiere M, Simonin K, Duval RE, Villedieu M, Leroy-Dudal J, Saison-Behmoaras E, Gauduchon P, Denoyelle C, Poulain L. Bcl-XL and MCL-1 constitute pertinent targets in ovarian carcinoma and their concomitant inhibition is sufficient to induce apoptosis. Int J Cancer. 2010; 126:885-895.
19. Haider T, Tiwari R, Vyas SP, Soni V. Molecular determinants as therapeutic targets in cancer chemotherapy: An update. Pharmacol Ther. 2019; 200:85-109.
20. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: Implications for physiology and therapy. Nat Rev Mol Cell Biol. 2014; 15:49-63.
21. Teixeira C, Reed JC, Pratt MA. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. Cancer Res. 1995; 55:3902-3907.
22. Zhang X, Huang Q, Yang Z, Li Y, Li CY. GW112, a novel antiapoptotic protein that promotes tumor growth. Cancer Res. 2004; 64:2474-2481.
23. Liu W, Liu Y, Li H, Rodgers GP. Olfactomedin 4 contributes to hydrogen peroxide-induced NADPH oxidase activation and apoptosis in mouse neutrophils. Am J Physiol Cell Physiol. 2018; 315:C494-C501.
24. Wang XY, Chen SH, Zhang YN, Xu CF. Olfactomedin-4 in digestive diseases: A mini-review. World J Gastroenterol. 2018; 24:1881-1887.
25. Lin Z, Yang S, Zhou Y, *et al*. OLFM4 depletion sensitizes gallbladder cancer cells to cisplatin through the ARL6IP1/caspase-3 axis. Transl Oncol. 2022; 16:101331.
26. Han Y, Liao Q, Wang H, Rao S, Yi P, Tang L, Tian Y, Oyang L, Wang H, Shi Y, Zhou Y. High expression of calreticulin indicates poor prognosis and modulates cell migration and invasion *via* activating Stat3 in nasopharyngeal carcinoma. J Cancer. 2019; 10:5460-5468.
27. Shi F, Shang L, Pan BQ, Wang XM, Jiang YY, Hao JJ, Zhang Y, Cai Y, Xu X, Zhan QM, Wang MR. Calreticulin promotes migration and invasion of esophageal cancer cells by upregulating neuropilin-1 expression *via* STAT5A. Clin Cancer Res. 2014; 20:6153-6162.
28. Ye J, Qi L, Du Z, Yu L, Chen K, Li R, Feng R, Zhai W. Calreticulin: A potential diagnostic and therapeutic biomarker in gallbladder cancer. Aging (Albany NY). 2021; 13:5607-5620.
29. Liu B, Liu Y, Zhao L, Pan Y, Shan Y, Li Y, Jia L. Upregulation of microRNA-135b and microRNA-182 promotes chemoresistance of colorectal cancer by targeting ST6GALNAC2 *via* PI3K/AKT pathway. Mol Carcinog. 2017; 56:2669-2680.
30. Chang Y, Liu C, Yang J, *et al*. MiR-20a triggers metastasis of gallbladder carcinoma. J Hepatol. 2013; 59:518-527.
31. Yang D, Zhan M, Chen T, Chen W, Zhang Y, Xu S, Yan J, Huang Q, Wang J. miR-125b-5p enhances chemotherapy sensitivity to cisplatin by down-regulating Bcl2 in gallbladder cancer. Sci Rep. 2017; 7:43109.
32. Zhang GF, Wu JC, Wang HY, Jiang WD, Qiu L. Overexpression of microRNA-205-5p exerts suppressive effects on stem cell drug resistance in gallbladder cancer by down-regulating PRKCE. Biosci Rep. 2020; 40:BSR20194509.
33. Lai X, Gupta SK, Schmitz U, Marquardt S, Knoll S, Spitschak A, Wolkenhauer O, Putzer BM, Vera J. MiR-205-5p and miR-342-3p cooperate in the repression of the E2F1 transcription factor in the context of anticancer chemotherapy resistance. Theranostics. 2018; 8:1106-1120.
34. Wang H, Zhan M, Xu SW, Chen W, Long MM, Shi YH, Liu Q, Mohan M, Wang J. miR-218-5p restores sensitivity to gemcitabine through PRKCE/MDR1 axis in gallbladder cancer. Cell Death Dis. 2017; 8:e2770.
35. Pelaz SG, Tabernero A. Src: coordinating metabolism in cancer. Oncogene. 2022; 41:4917-4928.
36. Hashemi M, Taheriazam A, Daneii P, Hassanpour A, Kakavand A, Rezaei S, Hejazi ES, Aboutalebi M, Gholamrezaie H, Saebfar H, Salimimoghadam S, Mirzaei S, Entezari M, Samarghandian S. Targeting PI3K/Akt signaling in prostate cancer therapy. J Cell Commun Signal. 2023; 17:423-443.
37. Bai J, Yang BJ, Luo X. Effects of 5-hydroxy-4'-nitro-7-propionyloxy-genistein on inhibiting proliferation and invasion *via* activating reactive oxygen species in human ovarian cancer A2780/DDP cells. Oncol Lett. 2018; 15:5227-5235.
38. White E. The role for autophagy in cancer. J Clin Invest. 2015; 125:42-46.
39. Qian X, Li X, Cai Q, *et al*. Phosphoglycerate kinase 1

phosphorylates Beclin1 to induce autophagy. Mol Cell. 2017; 65:917-931 e916.
40. Cai Q, Wang Z, Wang S, Weng M, Zhou D, Li C, Wang J, Chen E, Quan Z. Long non-coding RNA LINC00152 promotes gallbladder cancer metastasis and epithelial-mesenchymal transition by regulating HIF-1alpha *via* miR-138. Open Biol. 2017; 7:160247.
41. Cai Q, Wang S, Jin L, Weng M, Zhou D, Wang J, Tang Z, Quan Z. Long non-coding RNA GBCDRlnc1 induces chemoresistance of gallbladder cancer cells by activating autophagy. Mol Cancer. 2019; 18:82.
42. Chen C, Lu L, Yan S, Yi H, Yao H, Wu D, He G, Tao X, Deng X. Autophagy and doxorubicin resistance in cancer. Anticancer Drugs. 2018; 29:1-9.
43. Yalcin-Ozkat G. Molecular modeling strategies of cancer multidrug resistance. Drug Resist Updat. 2021; 59:100789.
44. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer. 2002; 2:48-58.
45. Hanssen KM, Haber M, Fletcher JI. Targeting multidrug resistance-associated protein 1 (MRP1)-expressing cancers: Beyond pharmacological inhibition. Drug Resist Updat. 2021; 59:100795.
46. Zhan M, Zhao X, Wang H, Chen W, Xu S, Wang W, Shen H, Huang S, Wang J. miR-145 sensitizes gallbladder cancer to cisplatin by regulating multidrug resistance associated protein 1. Tumour Biol. 2016; 37:10553-10562.
47. Hua H, Kong Q, Zhang H, Wang J, Luo T, Jiang Y. Targeting mTOR for cancer therapy. J Hematol Oncol. 2019; 12:71.
48. Li Q, Mou LJ, Tao L, Chen W, Sun XT, Xia XF, Wu XY, Shi XL. Inhibition of mTOR suppresses human gallbladder carcinoma cell proliferation and enhances the cytotoxicity of 5-fluorouracil by downregulating MDR1 expression. Eur Rev Med Pharmacol Sci. 2016; 20:1699-1706.
49. Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest. 2013; 123:3664-3671.
50. Zhan M, Wang H, Chen T, Chen W, Yang L, He M, Xu S, Wang J. NOX1 mediates chemoresistance *via* HIF1alpha/MDR1 pathway in gallbladder cancer. Biochem Biophys Res Commun. 2015; 468:79-85.
51. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, Sarkar S. Drug resistance in cancer: An overview. Cancers (Basel). 2014; 6:1769-1792.
52. Li Y, Tian M, Zhang D, Zhuang Y, Li Z, Xie S, Sun K. Long non-coding RNA myosin light chain kinase antisense 1 plays an oncogenic role in gallbladder carcinoma by promoting chemoresistance and proliferation. Cancer Manag Res. 2021; 13:6219-6230.
53. Jones BA, Varambally S, Arend RC. Histone methyltransferase EZH2: A therapeutic target for ovarian cancer. Mol Cancer Ther. 2018; 17:591-602.
54. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: An evolving paradigm. Nat Rev Cancer. 2013; 13:714-726.
55. Lopez-Serra L, Ballestar E, Fraga MF, Alaminos M, Setien F, Esteller M. A profile of methyl-CpG binding domain protein occupancy of hypermethylated promoter CpG islands of tumor suppressor genes in human cancer. Cancer Res. 2006; 66:8342-8346.
56. Wensheng L, Bo Z, Qiangsheng H, Wenyan X, Shunrong J, Jin X, Quanxing N, Xianjun Y, Xiaowu X. MBD1 promotes the malignant behavior of gallbladder cancer cells and induces chemotherapeutic resistance to gemcitabine. Cancer Cell Int. 2019; 19:232.
57. Sheng X, Sun X, Sun K, Sui H, Qin J, Li Q. Inhibitory effect of bufalin combined with Hedgehog signaling pathway inhibitors on proliferation and invasion and metastasis of liver cancer cells. Int J Oncol. 2016; 49:1513-1524.
58. Qian L, Su H, Wang G, Li B, Shen G, Gao Q. Anti-tumor activity of bufalin by inhibiting c-MET mediated MEK/ERK and PI3K/AKT signaling pathways in gallbladder cancer. J Cancer. 2020; 11:3114-3123.
59. Li C, Yang Z, Zhai C, Qiu W, Li D, Yi Z, Wang L, Tang J, Qian M, Luo J, Liu M. Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor alpha by inhibiting NF-kappaB signaling pathway. Mol Cancer. 2010; 9:73.
60. Yu Y, Wang J, Xia N, Li B, Jiang X. Maslinic acid potentiates the antitumor activities of gemcitabine *in vitro* and *in vivo* by inhibiting NF-kappaB-mediated survival signaling pathways in human gallbladder cancer cells. Oncol Rep. 2015; 33:1683-1690.
61. Gao H, Xie J, Peng J, Han Y, Jiang Q, Han M, Wang C. Hispidulin inhibits proliferation and enhances chemosensitivity of gallbladder cancer cells by targeting HIF-1alpha. Exp Cell Res. 2015; 332:236-246.
62. Wang FT, Wang H, Wang QW, Pan MS, Li XP, Sun W, Fan YZ. Inhibition of autophagy by chloroquine enhances the antitumor activity of gemcitabine for gallbladder cancer. Cancer Chemother Pharmacol. 2020; 86:221-232.
63. Wu WD, Hu ZM, Shang MJ, Zhao DJ, Zhang CW, Hong DF, Huang DS. Cordycepin down-regulates multiple drug resistant (MDR)/HIF-1alpha through regulating AMPK/mTORC1 signaling in GBC-SD gallbladder cancer cells. Int J Mol Sci. 2014; 15:12778-12790.
64. Wang H, Li X, Chen T, Wang W, Liu Q, Li H, Yi J, Wang J. Mechanisms of verapamil-enhanced chemosensitivity of gallbladder cancer cells to platinum drugs: Glutathione reduction and MRP1 downregulation. Oncol Rep. 2013; 29:676-684.
65. Huang S, Wang H, Chen W, Zhan M, Xu S, Huang X, Lin R, Shen H, Wang J. Tamoxifen inhibits cell proliferation by impaired glucose metabolism in gallbladder cancer. J Cell Mol Med. 2020; 24:1599-1613.
66. Slusarczyk M, Lopez MH, Balzarini J, Mason M, Jiang WG, Blagden S, Thompson E, Ghazaly E, McGuigan C. Application of ProTide technology to gemcitabine: A successful approach to overcome the key cancer resistance mechanisms leads to a new agent (NUC-1031) in clinical development. J Med Chem. 2014; 57:1531-1542.
67. McNamara MG, Bridgewater J, Palmer DH, Faluyi O, Wasan H, Patel A, Ryder WD, Barber S, Gnanaranjan C, Ghazaly E, Evans TRJ, Valle JW. A phase Ib study of NUC-1031 in combination with cisplatin for the first-line treatment of patients with advanced biliary tract cancer (ABC-08). Oncologist. 2021; 26:e669-e678.
68. Primrose JN, Fox RP, Palmer DH, *et al*. Capecitabine compared with observation in resected biliary tract cancer (BILCAP): A randomised, controlled, multicentre, phase 3 study. Lancet Oncol. 2019; 20:663-673.
69. Nakachi K, Ikeda M, Konishi M, *et al*. Adjuvant S-1 compared with observation in resected biliary tract cancer (JCOG1202, ASCOT): A multicentre, open-label, randomised, controlled, phase 3 trial. Lancet. 2023;

401:195-203.
70. Demols A, Borbath I, Van den Eynde M, Houbiers G, Peeters M, Marechal R, Delaunoit T, Goemine JC, Laurent S, Holbrechts S, Paesmans M, Van Laethem JL. Regorafenib after failure of gemcitabine and platinum-based chemotherapy for locally advanced/metastatic biliary tumors: REACHIN, a randomized, double-blind, phase II trial. Ann Oncol. 2020; 31:1169-1177.
71. Kim RD, Sanoff HK, Poklepovic AS, Soares H, Kim J, Lyu J, Liu Y, Nixon AB, Kim DW. A multi-institutional phase 2 trial of regorafenib in refractory advanced biliary tract cancer. Cancer. 2020; 126:3464-3470.
72. Sun W, Patel A, Normolle D, Patel K, Ohr J, Lee JJ, Bahary N, Chu E, Streeter N, Drummond S. A phase 2 trial of regorafenib as a single agent in patients with chemotherapy-refractory, advanced, and metastatic biliary tract adenocarcinoma. Cancer. 2019; 125:902-909.

Received March 1, 2023; Revised July 15, 2023; Accepted August 8, 2023.

[§]These authors contributed equally to this work.

*Address correspondence to:
Fei Ma, Department of Oncology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, No. 1665 Kongjiang Road, Shanghai 200092, China.
E-mail: mafei@xinhuamed.com.cn

Jun Ding, Department of Biliary and Pancreatic Surgery, Shanghai Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China.
E-mail: doctording0916@126.com

Released online in J-STAGE as advance publication August 17, 2023.