Roles of circRNAs on tumor autophagy

Wenming Cui, Qin Dang, Chen Chen, Weitang Yuan, and Zhenqiang Sun

INTRODUCTION

Circular RNAs (circRNAs) are a type of special noncoding RNA. circRNAs are highly stable and are found mainly in the cytoplasm. Most circRNAs are conserved and usually exhibit tissue specificity and timing specificity. In addition to the regulation mode of competitive endogenous RNA (ceRNA), circRNAs can also bind to RNA-binding proteins (RBPs), regulate alternative splicing, encode proteins or polypeptides, and regulate the expression of parent genes affecting biological pathways in which coded proteins are involved. Autophagy is an important cellular mechanism that plays an essential role in normal cell physiological processes and in diseases, especially tumors. Studies reported that circRNAs have an important effect on autophagic processes. What are the detailed biological functions and mechanisms of circRNAs in autophagy? In this article, we summarize the relationship between circRNAs and autophagy and the regulatory function and mechanism (especially as microRNA [miRNA] sponges and binding to RBPs) of circRNAs in autophagy. In addition, we discuss the dysregulation and functional and clinical applications of autophagy-associated circRNAs in a variety of diseases. Autophagy-associated circRNAs have the potential to be essential biomarkers of diagnosis and treatment and to be beneficial to the research and development of targeted drugs for tumor or non-tumor diseases.

circRNA
circRNAs are a type of endogenous noncoding RNA. circRNAs were first reported in plant viroids in 1971. In 1980, a study found that under an electron microscope, yeast mitochondrial RNA contained covalently closed circular molecules. Some researchers discovered in 1995 that the RNA transcript of the mouse testis determining gene Sry is circular. The formation of circRNA is closely related to the alternative splicing of exons and introns on pre-mRNA. Unlike traditional linear RNA, they have a circular closed structure formed by backsplicing without 3'-5' polarity. Recent studies have reported many ways in which circRNAs originate in cells (Figure 1). For example, many studies have proposed two models of circRNAs formation. One model is called lariat-driven circularization, and the other model is called intron-pairing-driven circularization. The lariat-driven circularization model indicates that pre-mRNA is folded during transcription. Adjacent exons are close, and exon skipping occurs. The splice donor and the splice acceptor combine to form a circRNA molecule intermediate in the folding region and then splice to form an exon-derived circRNA (Figure 1A). Additionally, researchers have identified that RBPs could form a bridge to promote the interaction of upstream intron and downstream intron and

types of cells. However, undernutrition or hypoxia may lead to an increase in autophagy levels. Autophagy can be divided into macroautophagy, microautophagy, and chaperone-mediated autophagy. Autophagy is closely related to many diseases, including many tumor diseases and nonneoplastic diseases. In addition, autophagy is also related to some of the body’s stress responses. In recent studies, we have increasingly found that circRNAs are closely related to autophagy, circRNAs regulate the occurrence and development of diseases by regulating the level of autophagy in various cells. In this article, we discussed the basic knowledge of circRNAs and autophagy and the regulatory relationship between the two. In particular, we summarized the currently known functional roles of autophagy-associated circRNAs in various diseases, as well as the important potential of autophagy-associated circRNAs as biomarkers in the diagnosis and treatment of related diseases.
promote the biological occurrence of exon-derived circRNA26 (Figure 1B). The intron-pairing-driven circularization points out that the intron region forms a ring structure through base-pairing. Further, through reverse splicing, exon-derived circRNAs or exon-intron-derived circRNAs are formed27,30 (Figure 1C). In addition to these two models, studies have also found a circRNA derived from introns28 (Figure 1D). Most introns will be degraded after splicing, and introns with a special consensus motif will not be degraded after splicing, thus forming intron-derived circRNA. The consensus motif contains a 7 nt GU-rich element (yellow box) near the 5’ splice site and an 11 nt C-rich element (red box) near the 3’ branch point site.28

Figure 1. The production process of circRNA in cells
(A) Lariat-driven circularization. Exon skips form a lariat, and the splice donor and splice acceptor are covalently spliced to form a circRNA molecular intermediate. Then the circRNA molecular intermediate is spliced again to form circRNA.25 (B) RBP-driven circularization. RBP binds to the upstream and downstream introns of circRNA to promoted its formation by removing introns.26 (C) Intron-pairing-driven circularization. Intron region forms a ring structure through base pairing. Then, exon-derived circRNAs or exon-intron-derived circRNAs are formed through reverse splicing.27 (D) Intronic circRNA. Introns with a special consensus motif will not be degraded after splicing, thus forming intron-derived circRNA. The consensus motif contains a 7 nt GU-rich element (yellow box) near the 5’ splice site and an 11 nt C-rich element (red box) near the 3’ branch point site.28

and development of the body and the occurrence and development of diseases through various mechanisms34,35 (Table 1). There are many kinds of circRNAs that play a role through various mechanisms, leading to specific biological functions. circRNAs can be used as competitive endogenous RNAs (ceRNAs) or miRNA sponges to regulate miRNA expression36 since the miRNA can bind to the 3’ untranslated region (3’ UTR) of mRNAs through the seed region, thereby degrading or inhibiting mRNA translation.41 Many studies have shown that some circRNAs bind to RBPs and thus play a biological function.37,42 Moreover, circRNAs are involved in the alternative splicing of mRNA.27,28,38 Although circRNA is a noncoding RNA, some recent studies have found that circRNA could also encode proteins or polypeptides.39,43 In addition, recent studies have found that circRNA may also regulate the degradation of miRNA through the mechanism of target-directed miRNA degradation (TDMD).40,44,45 TDMD is a mechanism to regulate the stability of miRNA.46 The target RNAs could be combined with miRNA and promote the 3’ end of miRNA to dislocate from the argonaute proteins (AGOs), binding and making it easier for enzymatic modifications.47,48 The 3’ end of miRNA is remodeled and degraded, thereby regulating the stability of miRNA.49 In addition to the above mechanisms, researchers have found that circRNAs could also participate in
regulating the expression of parental genes. An increasing number of studies have found that circRNAs play an important role in many diseases. In addition, circRNAs are stable in both intracellular and extracellular plasma, including blood and saliva. Thus, circRNAs can serve as biomarkers for disease diagnosis and prognosis, providing new potential therapeutic targets.

**Autophagy**

Autophagy is a highly evolutionarily conserved, dynamic process in eukaryotic organisms that degrades cellular components within the cell via lysosomes. Under basic conditions, low levels of autophagy exist in all types of cells. However, stimuli such as undernutrition or hypoxia may lead to an increase in autophagy levels. Current research indicates that autophagy is divided into three categories in mammalian cells, namely, macroautophagy, microautophagy, and chaperone-mediated autophagy (Figure 2). Among them, macroautophagy is the most important pathway. The whole process of autophagy is regulated by different autophagy-related proteins at all times. For example, the central molecule molecular target of rapamycin (mTOR) is a key protein that controls autophagy; mTOR can sense multiple changes in cells and enhance or reduce the level of autophagy. Studies have found that the occurrence of tumors may be closely related to disorders of autophagy. When autophagy functions normally, the tumor will be inhibited, but knocout autophagy genes will cause tumor formation. The effect of autophagy on tumor cells can be positive or negative. Autophagy prevents cells from becoming cancerous. However, when tumors are formed, autophagy greatly enhances the self-protection and metastatic ability of tumor cells. On the one hand, autophagy inhibits tumorigenesis and tumor development by eliminating oncoproteins and damaged organelles. On the other hand, autophagy helps tumor cells to overcome hypoxia and nutritional restrictions, thereby promoting tumor progression. In addition to its role in cancer, autophagy also plays an important role in some non-tumor diseases and stress reactions, such as silicosis and astrocyte activation.

**The relationship between circRNAs and autophagy**

Cell death is an important process in the growth and development of the body. Cell death is roughly divided into three categories, namely, apoptosis, autophagy, and necrosis. Different types of cell death occur through different signaling pathways. The relationship between circRNAs and apoptosis has been confirmed by some studies. For example, circ-Amotl1 reduces the apoptosis of myocardial cells and enhances the repair ability of the heart. circRNAs regulate cell death in a variety of ways, and their regulation of autophagy molecules is gradually being revealed. However, the relationship between circRNAs and autophagy and how circRNAs regulate autophagy remain unclear. In this section, we summarize the mechanisms by which circRNAs regulate autophagy and possible involved signaling pathways.

**The mechanism by which circRNAs regulate autophagy**

As a special type of nucleic acid, circRNA regulates biological functions in many ways. One of its important biological functions is to regulate autophagy. Regarding how to predict the relevant regulatory mechanisms, based on the current reports in the relevant literature, the changes of downstream autophagy molecules could be explored through high-throughput screening. Fully integrate bioinformatics could be as a basic tool, which could predict the potential interaction ability of nucleic acid and RNA, nucleic acid and protein for exploring the targeting molecules mediated by circRNA regulating autophagy. And then it may lay the foundation for the mechanism research methods of circRNA regulating autophagy. In the current literature reports, we found that some circRNAs could directly regulate autophagy-related genes to play an important role in the process of autophagy. For example, circ_0009910 could regulate the alternative splicing of circRNA, thereby promoting the classical splicing of linear RNA from the same parental gene, thereby exerting its biological function. The target RNAs could be encoded proteins or polypeptides, thereby regulating the level of protein expression. The target RNAs could be combined with miRNA and promote the 3′ end of miRNA to dislocate from the argonaute proteins (AGOIs), binding and making it easier for enzymatic modifications. The 3′ end of miRNA is remodeled and degraded, thereby regulating the stability of miRNA.

| Table 1. Different mechanisms of action of circRNAs |
|---|---|---|
| Number | Classification of mechanisms | Action form of mechanisms | Reference |
| 1 | miRNA sponges | circRNAs could be used as miRNA sponges to regulate miRNA expression, since the miRNA can bind to the 3′ UTR of miRNAs through the seed region, thereby degrading or inhibiting miRNA translation. | 36 |
| 2 | bind to RBP | circRNAs with RBP binding sites could bind to RBP, thereby regulating gene expression and exerting its biological functions. | 37 |
| 3 | regulate the expression of parental genes | Part of circRNAs could participate in regulating the transcription of its parent gene polymerase II, thereby regulating the expression of its parent gene. | 27 |
| 4 | regulate the alternative splicing | The reverse splicing of circRNA could compete with the classical splicing of linear RNA from the same parental gene, thereby exerting its function. | 38 |
| 5 | translation | Part of the circRNAs could encode proteins or polypeptides, thereby regulating the level of protein expression. | 39 |
| 6 | target-directed miRNA degradation | The target RNAs could be combined with miRNA and promote the 3′ end of miRNA to dislocate from the argonaute proteins (AGOIs), binding and making it easier for enzymatic modifications. The 3′ end of miRNA is remodeled and degraded, thereby regulating the stability of miRNA. | 40 |
circ_0035483 could regulate the expression of CCNB1 by sponging miR-335 to regulate autophagy. This is a question worthy of our continued discussion.

To date, the mechanism by which circRNAs regulate autophagy has remained controversial. Here, we clarify the regulatory relationship between the two (Table 2). Acting as miRNA sponges and directly binding to RBPs are important molecular mechanisms of circRNAs. On the one hand, circRNAs act as miRNA sponges or ceRNAs. There are many miRNA-binding sites on circRNAs that competitively bind miRNAs and affect the regulation of mRNAs by miRNAs. Studies have shown that circRNAs act as miRNA sponges and play an important regulatory role in autophagy. For example, hsa_circ_0035483 could sponge hsa-miR-335. When hsa-miR-335 was knocked down, the expression of hsa-miR-335 increased. hsa-miR-335 could be negatively correlated to target gene CCNB1. The expression of CCNB1 was reduced and autophagy was suppressed, thereby enhancing the sensitivity of gemcitabine in Renal clear cell carcinoma (RCC). Moreover, in research on Parkinson’s disease (PD), circDLGAP4 sponged miR-134-5p and inhibited the expression of miR-134-5p. cAMP-response element binding protein (CREB) is the target gene of miR-134-5p and could be inhibited by miR-134-5p. Thereby increasing the level of autophagy. In a study on epithelial ovarian cancer (EOC), circMUC16 could promote the expression of Beclin1 and RUNX1 by sponging miR-199a-5p. In addition to these, circMUC16 could also directly bind to the 475–526 region of ATG13 protein to promote the expression of ATG13. circMUC16 promoted autophagy in EOC by regulating Beclin1, RUNX1, and ATG13. Interestingly, RUNX1 could in turn promote the expression of circMUC16 by promoting transcription, thus forming a circular pathway.

On the other hand, studies have proved that circRNAs directly bind to RBPs to regulate autophagy. For example, DNA (cytosine-5-)-methyltransferase 3 beta (Dnmt3B) played a major role in the DNA methylation of PTEN-induced putative kinase 1 (Pink1). A circRNA called circ_006636 could directly bind to Dnmt3B and activate Pink1 expression by blocking the DNA methylation of Pink1 promoter. Pink1 could promote the phosphorylation of family with sequence similarity 65 member B (FAM65B). And phosphorylated FAM65B could inhibit autophagy of heart cells. circ_006636 regulated autophagy through the circ_006636-Pink1-FAM65B axis. In addition, in breast cancer, circ-DNMT1 directly binds to two proteins, p53 and Aadenine and Uracil (AU)-rich element RBP1 (AUF1) and promotes the nuclear translocation of these two nuclear proteins. Nuclear translocation of p53 induces cellular autophagy. AUF1 nuclear translocation improved the stability of DNA (cytosine-5-)-methyltransferase 1 (Dnmt1) mRNA, thus increasing Dnmt1 translation. Then the Dnmt1 protein entered the nucleus...
and inhibited p53 transcription through promoter methylation. circ-DNMT1 functioned by binding to p53 and AUF1, thereby enhancing autophagy in breast cancer. circPABPN1 binds to the RBP human antigen R (HuR) to enhance autophagy. HuR interacted with ATG16-like 1 (Atg16l1) mRNA via its 3' UTR to promote translation of Atg16l1. In addition, Atg16l1 plays an important role in the regulation of autophagy.84

These results imply that circRNAs are mainly involved in the regulation of autophagy by acting as mRNA sponges and directly binding to RBPs.

The signaling pathways via which circRNAs regulate autophagy

The relationship between circRNAs and autophagy is very strong and complex. Signaling pathway refers to a series of enzymatic reactions that can transfer molecular signals from outside the cell into the cell through the cell membrane to exert an effect.91 Signaling pathways play an important role in the physiological and pathological activities of cells.32,93 Some studies have shown that a variety of signaling pathways are involved in the process of circRNA regulation of autophagy and play an important role (Figure 4).

In esophageal squamous cell carcinoma (ESCC), the circRNA ciRS-7 has been used as a miR-1299 sponge to inhibit the autophagy of KYSE170 cells by targeting the epidermal growth factor receptor (EGFR)-AKT serine/threonine kinase (AKT)-mTOR signaling pathway.9 EGFR is an important transmembrane receptor.95 The AKT-mTOR pathway is located downstream of EGFR,96 and has a significant role in regulating autophagy.9 Moreover, the AMP-activated protein kinase (AMPK)-mTOR pathway has been shown to play an important role in the regulation of autophagy.97 In studies related to drug resistance in acute myeloid leukemia (AML), overexpression of circPAN3 promoted the progression of autophagy through the AMPK-mTOR signaling pathway.94 In a study on chronic myeloid leukemia (CML), circ_0009910 sponged miR-34a-5p, while miR-34a-5p directly targeted unc-51 like kinase 1 (ULK1). circ_0009910 promoted autophagy through this pathway to enhance imatinib resistance in CML.74 In addition to these, CREB pathway acted an important signaling pathway in PD.98 And CREB is a target gene of miR-134-5p. circDLGAP4 sponged miR-134-5p to enhance neuronal autophagy through this pathway to exert neuroprotective effects.91 In a recent study on diabetes peripheral neuropathy (DPN), autophagy-related circRNA (ACR) bound to miR-145-3p and then promoted the activation of the phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR pathway to inhibit cell autophagy.92 Therefore, circRNAs regulate autophagy through multiple signaling pathways to play essential biological functions.

Dual roles of circRNAs in autophagy regulation

Since there are many types of circRNAs and they have multiple biological functions, the mechanisms by which they regulate autophagy...
are diverse. The regulation of autophagy by circRNAs is based on the triggering of molecules related to the autophagy process to determine whether circRNAs could promote or inhibit autophagy. When circRNA acts on an autophagy activator, autophagy is activated. circRNA inhibits autophagy when acting on an autophagy inhibitor. Whether circRNAs promote or inhibit autophagy depends on the disease to be regulated and the state of the disease. From a broad perspective, circRNAs play a role in promoting autophagy and inhibiting autophagy in occurrence and development of disease (Figure 4).

On the one hand, circRNAs increase autophagy levels in cells. For instance, in drug-resistant RCC cells treated with gemcitabine, when hsa_circ_0035483 expression was downregulated, the LC3II/LC3I ratio was significantly reduced; that is, autophagy was inhibited. Additionally, circ-DNMT1 was proven to enhance the autophagy of breast cancer cells and promote breast cancer progression.

On the other hand, circRNAs inhibit cell autophagy. Recent studies showed that starvation or rapamycin-induced autophagy was inhibited by the overexpression of ciRS-7 in Eca109 cells. In a rat sciatic nerve injury model, downregulating the expression of circRNA.2837 induced neuron autophagy to reduce sciatic nerve injury. Interestingly, in a study related to lung cancer, circRNAs both promoted and inhibited autophagy in different lung cancer cell lines. In the serine/threonine kinase 11 (STK11) mutant cell lines A549 and H838, silencing circHIPK3-induced autophagy by reducing signal transducer and activator of transcription 3 (STAT3) phosphorylation and increasing 5’-AMPK catalytic subunit alpha (PRKAA) phosphorylation-related signaling. In the STK11 wild-type cell line H1299, silencing circHIPK3 inhibited autophagy mainly by reducing STK11-pPRKAA.

In summary, the regulation of autophagy by circRNAs is a complicated process. The mechanisms and related signaling pathways that we have summarized in the regulation of autophagy by circRNAs are far from enough, and we still need to continue to explore in the future.

**circRNAs regulate autophagy in cancers**

Recent studies have verified that circRNAs play an important role in the development and progression of tumors by regulating autophagy. circRNAs also regulate drug resistance during tumor chemotherapy by affecting autophagy.

RCC is the most common pathological type of kidney cancer and it often develops resistance during chemotherapy. Therefore, how to combat chemotherapy drug resistance is an important issue. One recent study discovered that hsa_circ_0035483 enhanced the resistance of RCC to gemcitabine and simultaneously promoted the autophagy induced by gemcitabine. Similarly, another study showed that in AML, overexpression of circPAN3 might at least partially upregulate autophagy through the AMPK-mTOR pathway to promote the development of AML resistance. In CML, circ_0009910 was highly expressed in the serum of patients with CML and CML cells. circ_0009910 promoted autophagy by sponging miR-34a-5p and enhanced the resistance of CML cells to imatinib. In a study related to drug resistance in thyroid cancer, the researchers found that circ_0060060 promoted cisplatin-induced autophagy, thereby inhibiting apoptosis and enhancing cisplatin resistance in papillary thyroid carcinoma (PTC) and anaplastic thyroid carcinoma (ATC) cells.

Cervical cancer patients have a high mortality rate due to metastasis and drug resistance. One article pointed out that hsa_circ_0023404
functioned as an miR-5047 sponge to enhance the chemoresistance of cervical cancer cells to cisplatin by regulating autophagy signaling.\(^7\)\(^9\)

In addition, in ESCC, the circRNA ciRS-7 was found to inhibit the progression of ESCC cells due to starvation or rapamycin-induced autophagy, thereby accelerating the progression of ESCC.\(^9\)\(^10\)\(^8\) Additionally, silencing of circHIPK3 significantly induced autophagy in STK11 mutant lung cancer cell lines (A549 and H838) via the MIR124-3p-STAT3-PRKAA-AMPK\(\alpha\) axis.\(^7\) Moreover, in breast cancer, a recent study indicated that circ-DNMT1 activated autophagy and promoted breast cancer progression by binding to and regulating the oncogenic proteins P53 and AUF1 in breast cancer cells.\(^10\) Matrine is a traditional Chinese medicine that can exert antitumor effects in a variety of cancers.\(^10\)\(^9\) Matrine inhibited the PI3K-AKT and Wnt-\(\beta\)-catenin pathways in glioma cells and downregulated the expression of circ-104075 to induce autophagy in the glioma cell line U251.\(^10\) In a study on EOC, circMUC16 could sponge miR-199a-5p to promote the expression of the target genes Beclin1 and RUNX1 of miR-199a-5p, thereby enhancing the autophagy level of EOC cells. The enhancement of autophagy increased the level of proliferation and invasion of EOC cells.\(^8\)\(^3\) Additionally, circ.0000515 could act as a sponge for miR-326 to promote the expression of the target gene ELK_1 of miR-326.\(^8\)\(^0\) At the same time, ELK_1 could inhibit the autophagy and apoptosis of cervical cancer cells, thereby increasing the proliferation and invasion levels of cervical cancer cells.

Thus, circRNAs play an essential role in regulating biological effects by affecting autophagy in multiple cancers. Autophagy-associated circRNAs may serve as key targets for tumor detection and treatment and open up new directions for future research on drug resistance of tumor chemotherapeutics.

**circRNAs regulate autophagy in non-cancers**

In addition to their role in cancer, autophagy-associated circRNAs also play a major role in some nonneoplastic diseases, such as in the lung fibrotic response in silicosis and sciatic nerve injury. Silicosis is one of the most common types of pneumoconiosis.\(^11\)\(^0\)\(^11\)\(^1\) In silica-treated lung fibroblasts, the activation of protein phosphatase 1 regulatory subunit 13B (PPP1R13B) was regulated by decreased circRNA-012091 in response to SiO\(_2\). Meanwhile, PPP1R13B promoted the proliferation and migration of lung fibroblasts by activating autophagy and endoplasmic reticulum stress (ERS), which led to the progression of silicosis.\(^19\) The sciatic nerve is the longest and thickest nerve in the body and the main nerve in the sacral nerve.\(^11\)\(^2\)\(^1\)\(^1\) One study reported that circRNA.2837, a member of the miR-34 family, acted as a sponge, and its downregulation induced neuronal autophagy to protect neurons from neurological damage and achieve protection in sciatic nerve injury.\(^12\)\(^1\)\(^1\) PD is a neurodegenerative disease commonly found in middle-aged and elderly people that has a substantial effect on patient quality of life.\(^11\)\(^4\) In MPTP-induced PD mouse models, the expression of circDLGAP4 was...
reduced. In vitro studies showed that circDLGAP4 exerted neuroprotective effects by enhancing cell autophagy and via other methods in PD.14

In addition to the diseases described above, some studies have demonstrated that autophagy regulation by circRNAs is also closely related to some stress responses in the body.15,16 Myocardial ischemia-reperfusion injury refers to the recanalization of the coronary artery after partial or complete acute obstruction at a certain time.15 Although the ischemic myocardium resumes normal perfusion, tissue damage in the area is a pathological process that progressively worsens.16 In a mouse model of myocardial ischemia-reperfusion injury, mmu_circRNA_006636 was demonstrated to directly bind to Dnmt3B and block Dnmt3B-mediated DNA methylation of the Pink1 promoter to activate Pink1 expression. In addition, Pink1 plays a role in inhibiting autophagy, thereby alleviating myocardial ischemia-reperfusion injury in mice.11 In addition, astrocytes were reported to be the most widely distributed cell in the mammalian brain, and they help to form the physical structure of the brain and actively play many roles.17 Downregulation of the circRNA HIPK2 inhibited astrocyte autophagy and astrocyte activation, as confirmed in mouse models.78 Similarly, another study on astrocyte activation found that reducing the expression of circHECTD1 inhibited autophagy in astrocytes, which led to inhibition of astrocyte activation in transient middle cerebral artery occlusion (tMCAO) mouse stroke models.77 Interestingly, circHECTD1 also played an important role in lung and pulmonary fibroblast activation.101 Moreover, Bcl-2/adenovirus E1B-19kDa-interacting protein 3 (BNIP3) is an important factor that regulates autophagy.118 circZNF29 inhibited autophagy of cardiomyocytes and reduced oxygen glucose deprivation (OGD)-induced cell damage by targeting BNIP3 to activate the mTOR signaling pathway.119

Thus, circRNAs play an essential role in regulating biological effects by affecting autophagy not only in cancers but also in some nonneoplastic diseases. The functions and potential applications of autophagy-associated circRNAs in non-tumor diseases and stress responses are worthy of affirmation and may be gradually demonstrated in future studies.

**Clinical applications of autophagy-associated circRNAs**

With the increased understanding of the relationship between circRNAs and autophagy, the clinical application potential of autophagy-associated circRNAs has been increasingly discovered.

Chemotherapy is a common treatment for cancer, but drug resistance often prevents chemotherapy from being effective, which makes tumor treatment difficult.120 Studies have reported that autophagy-associated circRNAs can be used as targets to decrease drug resistance in the treatment of cancer. Gemcitabine resistance is a frequent occurrence in the treatment of cancer.121 In RCC cells, hsa_circ_0035483 was more highly expressed in TK10 and UO31 cells treated with gemcitabine than in TK10 and UO31 cells treated without gemcitabine. In addition, hsa_circ_0035483 regulated CCNB1 to promote autophagy in RCC and enhance the resistance to gemcitabine by targeting hsa-miR-335. Therefore, hsa_circ_0035483 might be a target for therapy in gemcitabine-resistant RCC.8 Additionally, hsa_circ_0060060 might provide new ideas and therapeutic targets for the treatment of cisplatin resistance in human thyroid cancer cells.76 hsa_circ_0060060 promoted autophagy through the miR-144-3p-transforming growth factor α (TGF-α) axis, which enhanced cisplatin resistance in human thyroid cancer cells.76 At the same time, knocking out hsa_circ_0060060 enhanced the sensitivity of cisplatin. In acute myeloid leukemia (AML), circPAN3 regulates autophagy via AMPK-mTOR signaling to promote AML resistance.94 When circRNAs participate in the treatment of diseases through the miRNA sponge mechanism, miRNA antagonir may be more advantageous.122 miRNA antagonist is a special chemically modified miRNA antagonist. Through strong competitive binding with miRNA in the body, it has higher stability and inhibitory effect.123 In addition to acting as a miRNA sponge, circRNA may also play other unknown roles in the body. Antagomir can specifically bind to miRNA, prevent the complementary pairing of miRNA and its target gene mRNA, and inhibit miRNA from working. So as to achieve the purpose of treatment.

With the demonstration of the role of autophagy-associated circRNAs in tumors, they have become a promising marker for tumor prognosis and treatment (Figure 5). For example, circHIPK3 is an important autophagy regulator in non-small cell lung cancer.7 For patients with non-small cell lung cancer with non-stage I disease, the ratio between circHIPK3 and linHIPK3 (C:L ratio) might be an effective prognostic factor, and the survival rate of the high C:L ratio group was significantly lower than that of the low ratio group. In addition, autophagy-related circRNAs have also been used as targets for the treatment of various cancers. For example, in cervical cancer, hsa_circ_0000515 and hsa_circ_0023404 were highly expressed in cervical cancer tissues.79,80 When the expression of the circRNA hsa_circ_0000515 was reduced, cervical cancer cell proliferation and invasion were weakened, tumor growth was suppressed, and the autophagy level was enhanced.80 And hsa_circ_0023404 promoted the metastasis of cervical cancer and the progression of chemotherapy resistance.79 Thus, the combined assessment of hsa_circ_0000515 and hsa_circ_0023404 aided in diagnosing and determining the metastatic potential in patients with cervical cancer. Moreover, the expression of circMUC16 is upregulated in EOC tissues and serum of patients with EOC.83 At the same time, circMUC16 is closely related to the staging and classification of EOC. Therefore, circMUC16 may be a potential diagnostic and therapeutic target for EOC.

In addition to cancers, autophagy-associated circRNAs are also used as biomarkers and therapeutic targets in non-tumor diseases. It is well known that cerebral ischemic stroke seriously affects people’s health.124 One study pointed out that in tMCAO mouse stroke models, the expression of circHectd1 was significantly increased in ischemic brain tissue, and this result was verified in plasma samples from patients with acute ischemic stroke. Moreover, knocking down circHectd1 expression inhibited astrocyte autophagy and...
significantly reduced the infarct size in tMCAO mice. Therefore, circ HECTD1 is a kind of ACR that can be used as a new biological target and biomarker for stroke.

As a tumor marker, circRNA has higher stability and is an ideal non-invasive biomarker. In terms of treatment, circRNA has higher stability, a slower degradation rate, and a longer degradation time, which has more therapeutic advantages. At the same time, there are still some shortcomings worthy of our attention. For example, the expression of circRNA itself is relatively low, which affects the treatment efficiency to a certain extent. The use of autophagy-associated circRNAs as tumor markers and therapeutic targets is a process of research and development and gradual exploration. As mentioned above, autophagy-associated circRNAs have the potential to be used as drug sensitivity markers, tumor prognosis markers, diagnostic markers, and therapeutic targets for tumor drug resistance in the future.

Conclusions
With the progress of circRNA research, the mechanism by which circRNAs regulate autophagy is gradually being discovered. circRNAs have been found to play a significant role in the occurrence and development of tumors, resistance to chemotherapy drugs, and regulation of the body’s stress response through the regulation of autophagy. Because of the stability and tissue specificity of autophagy-associated circRNAs, they are stable in both intracellular and extracellular plasma, including blood and saliva. This implies their potential application in clinical diagnosis and treatment. They may be more accurate to use as biomarkers for cancer than current tumor markers. However, what role do circRNAs play in autophagy during all stages of the disease process? As tumor biomarkers, what are the sensitivity and specificity values of circRNAs? These issues are essential and need to be further addressed. With an understanding of the detailed mechanism by which circRNAs regulate autophagy, autophagy-associated circRNAs will have superior prospects as drug sensitivity markers, tumor prognosis markers, diagnostic markers, and therapeutic targets for tumor drug resistance in the future.

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Z.S. and W.Y. provided direction and guidance throughout the preparation of this manuscript. W.C. wrote and edited the manuscript. C.C. reviewed and made significant revisions to the manuscript. Q.D. collected and prepared the related papers. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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