Review

Long non-coding RNAs involved in autophagy regulation

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Autophagy degrades non-functioning or damaged proteins and organelles to maintain cellular homeostasis in a physiological or pathological context. Autophagy can be protective or detrimental, depending on its activation status and other conditions. Therefore, autophagy has a crucial role in a myriad of pathophysiological processes. From the perspective of autophagy-related (ATG) genes, the molecular dissection of autophagy process and the regulation of its level have been largely unraveled. However, the discovery of long non-coding RNAs (lncRNAs) provides a new paradigm of gene regulation in almost all important biological processes, including autophagy. In this review, we highlight recent advances in autophagy-associated lncRNAs and their specific autophagic targets, as well as their relevance to human diseases such as cancer, cardiovascular disease, diabetes and cerebral ischemic stroke.

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Facts

- Autophagy degrades non-functioning or damaged components of cells to maintain cellular homeostasis and thus has a very significant role in cell development, differentiation and death.
- LncRNAs have emerged as non-canonical regulators that take part in a collection of pathophysiological processes, including autophagy, by directly binding to RNA, DNA or protein.
- LncRNAs generally modulate autophagy via regulating the expression of ATG genes. They often function as competing endogenous RNAs (ceRNAs) to modulate autophagy-related microRNAs (miRNAs).
- Autophagy may, in turn, regulate lncRNA expression, although only one such lncRNA has been found so far.

Open Questions

- Can autophagy-related lncRNAs directly regulate ATG genes apart from indirect modulation via miRNAs or epigenetic modification enzymes, and if so, how?
- Among the several ATG genes that are simultaneously regulated by one autophagy-related lncRNA, is there a chief gene that determines the occurrence or repression of autophagy?
- How does autophagy regulate the levels of lncRNAs? Does it degrade them directly in a selective manner or regulate them indirectly?

LncRNAs are non-coding RNAs longer than 200 nucleotides.1,2 They have recently been found to have a crucial role in various fundamental physiopathologic processes, such as carcinogenesis, as well as autoimmune, cardiovascular and neurological diseases.3-5 LncRNAs can be classified according to their gene locations or functions. On one hand, lncRNAs can be named after their gene loci relative to adjacent protein-coding genes, which are antisense lncRNAs, intronic lncRNAs, bidirectional lncRNAs and intergenic lncRNAs (also named lincRNAs).6-9 On another, lncRNAs can act as decoys, scaffolds, guides and enhancers to regulate DNA, RNA and proteins, on the basis of their function.10-14 In addition, lncRNAs have recently been documented to serve as competing endogenous RNAs (ceRNAs), which can sequester microRNAs (miRNAs) from targeted mRNAs sharing the same microRNA response elements (MREs), thereby regulating the expression of the targeted mRNAs.15 Owing to the increasing number of functionally characterized lncRNAs, the classification is constantly being updated. In this review, we will summarize the functions of lncRNAs involved in autophagy, a self-digestive process that helps to maintain cellular homeostasis.16,17

Macroautophagy (hereafter referred to as autophagy) is a process that delivers cytoplasm components, enclosed in double-membrane vesicles, to lysosomes for degradation.16 By doing so, autophagy has a critical role in maintaining cellular homeostasis in response to various environmental stresses, such as nutrient deprivation and hypoxia, as well as chemical and physical damage.16,17 Therefore, autophagy is crucial in various pathological and physiological processes such as immunity, cancer, cardiovascular diseases and neurodegenerative diseases.18-20

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To date, at least 37 autophagy-related (ATG) genes are known to exist in yeast, and many of their orthologs have been identified in mammals.21,22 We will discuss the crosstalk between autophagy-related lncRNAs and various ATG genes based on the four well-defined steps of autophagy: initiation, phagophore nucleation, autophagosome elongation/closure and autolysosome fusion23 (Figure 1). Certainly, lncRNAs may target several ATG genes at the same time and thus regulate autophagy at different stages. In this case, we will classify them into the category of most relevant stage.

LncRNAs Involved in Regulating Autophagy via ATG Genes

LncRNAs related to autophagy initiation. Adenine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin complex 1 (mTORC1) are two major proteins that sense external stimuli for autophagy initiation.24–26 Upon energy limitation, AMPK is phosphorylated and activated by increased AMP to inhibit mTORC1 and to activate the ATG1/ULK1/2 (yeast/mammal) complex, leading to the initiation of autophagy.24–26 In addition, mTORC1 can be activated by class I phosphatidylinositol 3-kinase (PI3K)/AKT signaling, and thus the PI3K/AKT/mTOR pathway is negatively linked to autophagy initiation.27,28 Once autophagy is initiated, ATG17 forms a complex with ATG29 and ATG31, and then interacts with ATG1 and ATG13 to mediate the assembly of pre-autophagosomal structure (PAS), (the site of autophagosome formation) in yeast.29,30 Similarly, in mammalian cells, ULK1/2 (mammal ortholog of ATG1) forms a stable complex with ATG13, FIP200/RB1CC1 and ATG101 and is subsequently transferred to omegasomes (the cradle for autophagosome biogenesis).31,32

A study reveals that high glucose reduces the level of lncRNA H19, which subsequently relieves the transcriptional repression of DIRAS3, and ultimately induces autophagy initiation by repressing PI3K/AKT/mTOR pathway.33 As marked activation of autophagy by high glucose has been shown to be detrimental to cardiac function, overexpression of H19 helps to alleviate this tendency.33 In addition, downregulation of H19 can increase Beclin 1 (mammal orthologous of ATG6) and ATG7 expression, which is a good indicator for further investigation of the association between H19 and autophagy.33,34 Contrarily, another study has found that exogenous overexpression of H19 induces autophagic cell death in cerebral ischemia and reperfusion (I/R) injury.34 Researchers confirmed that H19 induces autophagy by inhibiting DUSP5, a mitogen-activated protein kinase phosphatase. DUSP5 is known to repress phosphorylation of ERK1/2, and activation of the latter has been reported to provoke autophagy.35 The opposite effects of H19 on autophagy in diabetic cardiomyopathy and cerebral I/R injury indicate that conditional gene interference with H19 may be an efficient therapeutic approach to different pathological processes.

A recent study suggests that the lncRNA neighbor of BRCA1 gene 2 (NBR2) can bind to AMPK and promote its activation. Intriguingly, the expression of NBR2 can be induced by the increasing activation of AMPK under energy stress. Thus, AMPK/NBR2 forms a feed-forward loop to sustain AMPK activation in response to energy stress, which is a protective factor for normal cells to resist tumors.36,37 Indeed, NBR2 expression is reduced in human cancers, and loss of its function is correlated with poor prognosis for cancer patients.36,38,39 This study serves as an interesting example illustrating that lncRNAs can regulate protein activation through direct binding. Future work is needed to demonstrate this functional model of lncRNAs and to address how it takes effect: through changing the conformation of the protein or altering its affinity for other regulatory factors? Another study demonstrates that miR-19a can negatively regulate NBR2 and AMPK, probably by base pairing between miR-19a and NBR2.
or miR-19a and PRAA1 (the gene encoding AMPK).\(^{40}\) The upregulation of miR-19a in acute liver failure (ALF) results in the downregulation of NBR2 and AMPK expression, which represses autophagy. In light of the protective role of autophagy in the progression of ALF, activation of the miR-19a-NBR2/AMPK regulatory axis exacerbates ALF.\(^{40}\) This study gave rise to the hypothesis of a ceRNA network in which NBR2 might act as a sponge for miR-19a and prevent it from binding to AMPK mRNA.

As a multitargeted tyrosine kinase inhibitor (TKI), sorafenib has been demonstrated to induce autophagic hepatic carcinomas (HCC) cell death, and thus the blockage of autophagy facilitates sorafenib resistance in HCC.\(^{41,42}\) Several miRNAs, including miR-21, miR-216a, miR-217 and miR-494, have been shown to confer sorafenib resistance in HCC by inhibiting autophagy through targeting phosphatase and tension homolog (PTEN).\(^{43-45}\) For the sake of repressing sorafenib resistance, an artificial long non-coding RNA (AlncRNA), Ad5-AlncRNA, was constructed to target these miRNAs simultaneously.\(^{45}\) Overexpression of Ad5-AlncRNA in HCC can sequester these miRNAs from binding to the 3'-UTR of PTEN mRNA. As a result, PTEN is upregulated to repress AKT/mTOR activity and then activates autophagy, sequentially reversing sorafenib resistance.\(^{45}\) Thus, artificial lncRNAs targeting several miRNAs of the same mRNA will be a potent therapeutic strategy for diseases.\(^{45,46}\)

IFN-γ induced by Mycobacterium bovis BCG can repress lncRNA maternally expressed gene 3 (MEG3), which decreases p70-S6K (Thr389) phosphorylation subsequently, a downstream factor of mTOR complex, indicating mTOR inactivation. As a result, autophagy is activated and eradication of intracellular Mycobacterium bovis BCG is enhanced.\(^{47}\) Similar to pathogen infection in macrophages, tumor cells may also trigger autophagy to survive under various stresses through repressing MEG3. Indeed, the MEG3 expression level is significantly reduced in bladder cancer cells, leading to increased autophagy flux and cell proliferation.\(^{48}\) In addition, MEG3 has also been found to promote cisplatin-induced apoptosis by inhibiting autophagy in human glioma cells.\(^{49}\) By contrast, another study shows that overexpression of MEG3 induces autophagy, thereby repressing tumorigenesis and progression of epithelial ovarian carcinoma by regulating ATG3. MEG3 can bind to ATG3 protein and protect ATG3 mRNA from degradation.\(^{50}\) However, more work is needed to elucidate how MEG3 modulates ATG3 protein and mRNA.

Studies have demonstrated that downregulation of the lncRNA HOTAIRM1 can block autophagy, and thus inhibit all-trans retinoic acid (ATRA)-induced autophagic degradation of PML-RARA in promyelocytic leukemia (PML) cells.\(^{51-53}\) HOTAIRM1 may provoke autophagy by preventing miR-20a/106b and miR-125b from decreasing ULK1, E2F1 and DRAM2 expression, thus contributing to autophagy-dependent degradation of PML-RARA.\(^{53-56}\) This indicates that overexpression of HOTAIRM1 might be a potential therapeutic measure for PML.

The lncRNA PTENP1 shares a similar 3'-UTR with the tumor-suppressor gene PTEN, and protects PTEN from miRNA-mediated silencing.\(^{57}\) Sustained PTENP1 expression in HCC cells upregulates PTEN expression and suppresses the PI3K/AKT signaling cascade, which results in the induction of autophagy, as well as the inhibition of cell proliferation and migration/invasion.\(^{58}\) In addition, PTENP1 can antagonize miR-17 and miR-20a to enhance the expression of ULK1, ATG7 and p62/SQSTM1 (sequestosome 1).\(^{58-60}\) Furthermore, overexpression of PTENP1 can suppress mTOR phosphorylation and downregulate Bcl-2 expression.\(^{58}\) Nevertheless, it remains to be determined whether molecules in this complicated regulatory network are directly regulated by PTENP1 or indirectly altered secondary to some initial factors. Interestingly, researchers have constructed Sleeping Beauty (SB)-based hybrid baculovirus (BV) vectors for sustained PTENP1 expression.\(^{58}\) This system could be a promising instrument for endogenous overexpression of IncRNAs in specific tissues for therapeutic purposes.

The IncRNA regulator of insulin sensitivity and autophagy (Risa) can affect insulin sensitivity by altering autophagic activity. Indeed, knockdown of Risa increases the phosphorylation of ULK1 (Ser757), which contributes to autophagy activation and attenuates insulin resistance.\(^{61}\) Unfortunately, there are still divergent schools of thought regarding what role autophagy has in insulin resistance or diabetes.\(^{62,63}\) Thus, whether knockdown of Risa can alleviate insulin resistance by promoting autophagy in patients with diabetes requires further exploration.

The lncRNA AK156230 has been found to repress replicative senescence (RS) in mouse embryonic fibroblasts (MEFs), and one of the downstream pathways involved is autophagy.\(^{64}\) Pathway analysis shows that the mTOR signaling pathway is associated with AK156230 knockdown, and transcriptional levels of ATG genes including ULK2, ATG7 and ATG16L apparently decrease accordingly.\(^{64}\) Vague though the facts are, AK156230 seems to participate in autophagy induction, as its downregulation results in a deficiency of autophagy, which may accelerate aging.\(^{65}\)

Another lncRNA, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), has attracted a great deal of interest for its elusive role in autophagy regulation.\(^{66-71}\) MALAT1 is upregulated and acts as a sponge for miR-26b to upregulate its target ULK2 in cerebral I/R injury.\(^{71}\) In light of the protective effect of autophagy against I/R in brain microvascular endothelial cells, the enhanced activity of the MALAT1/miR-26b/ULK2 regulatory axis appears to be a self-defense mechanism in response to ischemic stroke, the pathological basis of which is I/R injury.\(^{71}\) Another study demonstrates that MALAT1 silencing notably elevates p62 and decreases LAMP2 expression, which is essential for the fusion of autophagosomes and lysosomes.\(^{66}\) Moreover, MALAT1 can act as a ‘sponge’ for miR-216b to induce autophagy, probably through derepressing Beclin 1 expression, which, in consequence, ameliorates the multidrug resistance of HCC cells.\(^{67,68}\) Interestingly, a newly published article reports that MALAT1 knockdown can indeed reduce the expression of Beclin 1 in multiple myeloma tissues, although the details of the mechanism are not known.\(^{69}\) In contrast to the studies mentioned above, MALAT1 inhibition has been found to induce autophagy in diffuse large B-cell lymphoma (DLBCL) cells and improve its response to adriamycin treatment.\(^{70}\)

As summarized in Figure 2 and Table 1, the lncRNA NBR2 promotes autophagy initiation by directly activating AMPK.\(^{36}\) The lncRNAs Ad5-AlncRNA\(^{45}\) and PTENP1\(^{58}\) provoke
Autophagy-related long non-coding RNAs

**Figure 2** Brief summarization of the initiation step of mammalian core autophagy machinery and its regulation by lncRNAs. Upon energy limitation, autophagy can be initiated by AMPK and mTORC1. Then, ULK1 complex composed of ULK1, ATG13, FIP200 and ATG101 is activated. IncRNA NBR2 directly modulates AMPK and mTORC1 are positively regulated by MEG3 and H19 and negatively regulated by Ad5-AlncRNA. In addition, IncRNA PTENP1, Risa, MALAT1, TGFB2-OT1, AK156230 and HOTAIRM1 collectively regulate ULK1 complex.

autophagy initiation by repressing the PI3K/AKT/mTOR pathway, whereas MEG3 is positively regulated by AMPK and mTORC1. MEG3 and H19 have the opposite effect. The IncRNAs HOTAIRM1, PTENP1 and MALAT1 enhance autophagy initiation by increasing ULK expression. Meanwhile, the IncRNA Risa negatively regulates autophagy initiation by inhibiting ULK1 activation. In addition, the IncRNAs H19, MEG3, AK156230, PTENP1, and MALAT1 can also influence other ATG genes and autophagy adaptor proteins involved in later steps of autophagy regulation.

**LncRNAs related to phagophore nucleation.** Once activated and translocated to PAS/omegasome, the ATG1/ULK1 complex can activate the class III PI3K complex, which mainl comprises Vps34, Vps15, vps30/Becn1 (yeast/mammal) and ATG14/Barkor (yeast/mammal), to generate phosphatidylinositol 3-phosphate (PI3P). PI3P recruits double FYVE-containing protein 1 (DFCP1) to promote the formation of the omegasome. Meanwhile, other essential regulators such as ATG9, ATG18 (WIP11/2/3/4) and VMP1 are present on the autophagic membrane. During the process of class III PI3K complex formation and function, BCL-2 and Rubicon act as two negative regulators.

Two studies have demonstrated that the inhibition of LincRNA, regulator of reprogramming (Linc-ROR) can elicit autophagy by upregulating Beclin 1 expression, and revise gemcitabine and tamoxifen resistance in breast cancer respectively. However, further experiments need to reveal how Linc-ROR regulates Beclin 1 expression and whether knockdown of Linc-ROR could be feasible in clinical practice.

LncRNA loc146880 reveals to be intrinsically related to autophagy and the biogenesis of lung cancer. High expression of reactive oxygen species (ROS) induced by PM2.5 (a class of particulate matters, <2.5 μm in diameter) exposure enhances IncRNA loc146880 expression, which results in autophagy activation and subsequently contributes to lung cell migration and invasion. Beclin 1 mRNA is upregulated along with the increase of loc146880 expression, but the underlying mechanism is far from fully explained.

LncRNA AC023115.3 is upregulated by cisplatin and promotes cisplatin-induced apoptosis by impeding autophagy in glioma. Further mechanistic studies reveal that AC023115.3 can elevate GSK3β expression by sponging miR-26a. GSK3β promotes the degradation of Mcl1, a member of BCL-2 family that sequesters Beclin 1 from the class III PI3K complex. Further studies are needed to address whether Beclin 1 will activate the class III PI3K complex to a greater extent upon overexpression of AC023115.3 as hypothesized.

Regardless, the study reveals that the AC023115.3/miR-26a/GSK3β signaling cascade has a significant role in promoting chemosensitivity in gliomas and serves as an exciting indicator to exploit the association between AC023115.3 and Beclin 1.

Collectively, both Linc-ROR and loc146880 can impact vesicle nucleation by modulating Beclin 1 gene or protein expression (Figure 3; Table 1). In addition, the association between AC023115.3 and Beclin 1 still needs further investigation.

**InRNAs related to autophagosome elongation/closure.** The two unique ubiquitin-like conjugation systems have crucial roles in the elongation and closure of the isolated membrane. Driven by ATG7 (E1-like enzyme) and ATG10 (E2-like enzyme), ATG12 conjugates to ATG5 and then interacts with ATG16 (mammal ortholog is ATG16L) to form the ATG12-ATG5-ATG16 complex. Subsequently, the ATG12-ATG5-ATG16 complex, ATG7 and ATG3 (E2-like enzyme) jointly transform ATG8 (LC3 in mammals) to its cytosolic soluble isoform (LC3-I) to its membrane-anchored isoform (LC3-II). In addition, adaptor proteins such as ATG19 (an adaptor for the Cvt pathway) and ATG32 in yeast, as well as the neighbor of BRCA1 gene 1 (NBR1), Nix (also called Bnip3L) and p62 in mammals, selectively mediate the degradation of proteins or organelles by recruiting them to autophagosomes via binding to LC3-II.

A study reveals that the LncRNA TGFB2 overlapping transcript 1 (TGFB2-OT1, also known as FLJ11812) can be upregulated by vascular endothelial cell (VEC) inflammation triggers and function as the sponge for miR-3960, miR-4488 and miR-4459, thereby increasing the expression levels of their targets, such as ATG13, ceramide synthase 1 (CERS1) and La ribonucleoprotein domain family, member 1 (LARP1). In addition, overexpression of TGFB2-OT1 can also increase ATG3, ATG7 and p62 expression, probably through upregulating LARP1 by sponging miR-4459, an RNA-binding protein related to transcript stability and translation of mRNAs. Moreover, when TGFB2-OT1 prevents miR-3960-mediated repression of CERS1, production of C18-ceramide is
The IncRNA growth arrest-specific 5 (GAS5) has been reported to inhibit autophagy and enhance cisplatin sensitivity in NSCLC cells. In contrast to its deletion in several species of tumors, GAS5 is upregulated in osteoarthritis (OA), repressing autophagy and stimulating the apoptosis of OA chondrocytes, which is a key determinant responsible for cartilage degradation and thus OA pathogenesis. High expression of GAS5 in OA represses autophagy possibly through downregulating Beclin 1, ATG3, ATG5, ATG12 and LC3B expression. It seems that GAS5-repressed autophagy is beneficial for enhancing drug sensitivity but results in the occurrence of OA. Therefore, different modes of interference with GAS5 may be essential for distinct therapeutic purposes.

The IncRNA HNF1A-AS1 can sequester miR-30b from binding to its target ATG5 and thereby provoke autophagy in HCC. In addition, Beclin 1 and ATG12 have also been defined as targets of miR-30b in a previous study, indicating that, aside from ATG5, HNF1A-AS1 might also upregulate Beclin 1 and ATG12 expression to promote vesicle nucleation and autophagosome elongation/closure. Considering that autophagy was previously confirmed to promote HCC, it is likely that HNF1A-AS1 facilitates HCC biogenesis by promoting autophagy. Exogenous overexpression of the IncRNA, prostate cancer gene expression marker 1 (PCGEM1), can increase the mRNA levels of Beclin 1, ATG3, ATG5 and ATG12; indicating that PCGEM1 may be involved in the induction of autophagy, and promote the proliferation of human synoviocytes. PCGEM1 expression is associated positively with mortality rate in African-American patients with prostate cancer, probably because of PCGEM1-induced autophagy. However, further analysis is required to confirm whether and how PCGEM1 affects autophagy.

Mounting evidences reveal that LncRNAs harbor much more tissue and developmental stage specificity than coding transcripts. In line with this discovery, IncRNA highly increased to induce mitophagy (a class of selective autophagy targeting dysfunctional mitochondria) by directly interacting with LC3-II-containing autopagolysosomes upon Drp1-dependent mitochondrial fission. In summary, ectopic expression of TGFβ2-OT1 induced by VEC inflammation triggers activates autophagy via increasing ATG7, ATG3, ATG7 and p62 expression, and a small molecular inhibitor 3BDO significantly decreases TGFβ2-OT1 level and inhibits the subsequent autophagic and inflammatory reaction. Given that autophagy and inflammation have an intricate correlation, intervening in TGFβ2-OT1 could be a possible treatment strategy for infectious and autoimmune diseases.

### Table 1: IncRNAs involved in autophagy regulation

| Autophagy stages | Relevant autophagy-related genes or proteins | IncRNA |
|------------------|--------------------------------------------|--------|
| Initiation       | AMPK, mTORC1, ATG13, ULK1/2                | NBR2, GAS5, Chast |
|                  |                                            | Ad5-AlncRNA, MEG3, AK156230 |
|                  |                                            | PTENP1, PTEN, FLJ11812 |
|                  |                                            | MALAT1, Linc-ROR |
|                  |                                            | HOTAIR, PCGEM1, MALAT1 |
|                  |                                            | PTENP1, H19, MALAT1 |
| Vesicle nucleation| Beclin 1                                   |        |
| Autophagosome elongation/closure | Bcl-2, ATG7, ATG5, ATG3, ATG12, ATG16L, p62, LAMP2 | Beclin 1, PCGEM1, MALAT1 |
| Fusion           | Plekhm1, LAMP2                             |        |

Figure 3: Brief summarization of the vesicle nucleation step of mammalian core autophagy machinery and its regulation by IncRNAs. Vesicle nucleation is predominantly modulated by class III PI3K complex, which comprises Vps34, Vps15, Beclin 1 and ATG14/ATG14L. During the process of PI3K complex formation and function, BCL-2 and Rubicon act as two negative regulators. IncRNA GAS5, PCGEM1 and Linc-ROR regulate PI3K complex, among which, Beclin 1 itself is negatively regulated by IncRNA PTENP1. Moreover, IncRNA PTENP1 can repress autophagy via increasing BCL-2 expression.

The IncRNA growth arrest-specific 5 (GAS5) has been reported to inhibit autophagy and enhance cisplatin sensitivity in NSCLC cells. In contrast to its deletion in several species of tumors, GAS5 is upregulated in osteoarthritis (OA), repressing autophagy and stimulating the apoptosis of OA chondrocytes, which is a key determinant responsible for cartilage degradation and thus OA pathogenesis. High expression of GAS5 in OA represses autophagy possibly through downregulating Beclin 1, ATG3, ATG5, ATG12 and LC3B expression. It seems that GAS5-repressed autophagy is beneficial for enhancing drug sensitivity but results in the occurrence of OA. Therefore, different modes of interference with GAS5 may be essential for distinct therapeutic purposes.

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upregulated in liver cancer (HULC) has been found to predominantly express in primary HCC and metastatic hepatic carcinoma. One study elucidates clearly how the ‘HULC/USP22/Sirt1/protective autophagy’ pathway attenuates the chemosensitivity of HCC. In detail, HULC can upregulate ubiquitin-specific peptidase (USP22) expression level via repressing miR-6825-5p, miR-6845-5p and miR-6886-3p, and ectopic expression of USP22 can stabilize Sirt1 protein, which promotes protective autophagy by deacetylating Atg5 and Atg7. Mazy as it seems, this HULC-regulated autophagy pathway makes a promising target to address chemoresistance in HCC. Another work indicates that HULC overexpression increases proliferation and invasion of gastric cancer (GC) cells probably through arousing autophagy activation. Clearly, these findings put forward a new topic worthy of studying that how HULC provokes autophagy in GC and whether HULC can be identified as a biomarker both in HCC and GC.

In anoxia/reoxygenation (A/R)-induced autophagy of cardiomyocytes, the lncRNA autophagy promoting factor (APF) is upregulated to protect ATG7 from being downregulated by miR-188-3p, and thereby promotes autophagic death of cardiomyocytes. Intriguingly, despite the poor conservation of full-length APF across species, the binding site for miR-188-3p is highly conserved, highlighting the significance of the APF/miR-188-3p/ATG7 regulatory axis in autophagy activation.

The lncRNA HOTAIR is well characterized for recruiting the epigenetic modification factors, such as polycomb repressive complex 2 (PRC2), to regulate gene expression, thereby promoting tumor cell proliferation and migration. Analogously, HOTAIR is upregulated in chondrosarcoma and induces DNA methylation of miR-454-3p promoter regions by recruiting EZH2 and DNA methyltransferase 1 (DNMT1), which markedly silences miR-454-3p expression. ATG12 and STAT3 are targets of miR-454-3p, providing one molecular dissection of HOTAIR deficiency-induced autophagy repression and apoptosis. Another study demonstrates that HOTAIR is upregulated in HCC to promote HCC cell proliferation, probably by enhancing ATG3 and ATG7 expression to expedite autophagy flux. As HOTAIR can interact with numerous miRNAs, such as miR-34a, miR-331-3p, miR-130a and miR-454-3p, we should recall that HOTAIR regulates autophagy in two ways. First, HOTAIR may serve as a scaffold to recruit epigenetic modification enzymes to inhibit miRNA transcription; second, HOTAIR can act as a sponge to sequester miRNAs from their targets. In both cases, HOTAIR will upregulate specific genes targeted by corresponding miRNAs, possibly including some ATG genes. For instance, miR-130a can repress the transcription of ATG5 and ATG16L, and Atg12 is a target of miR-454-3p. 

Taken together, as shown in Figure 4 and Table 1, the lncRNAs TGFB2-OT1, GAS5, HNF1A-AS1, PCGEM1, HULC, APF and HOTAIR promote autophagosome elongation/closure by elevating the expression of ATG genes involved in the ubiquitin-like conjugation systems. Meanwhile, the lncRNA GAS5 may repress and the lncRNA PCGEM1 may activate autophagy nucleation by interacting with Beclin 1. The lncRNA TGFB2-OT1 activates autophagy initiation by increasing ATG13 expression.

**LncRNAs related to autolysosome fusion.** The final step of autophagy flux is the fusion of the autophagosome and the lysosome to form an autolysosome, where the autophagic cargo is degraded. The core molecules in this stage include the Rab-SNARE system and the lysosome membrane proteins LAMP1 and LAMP2. In addition, adaptor proteins are necessary to link endocytic and autophagic pathways to the lysosome. Pleckstrin homology domain-containing protein family M member 1 (Pleckhm1) is one such adaptor protein, which contains an LGS-interacting region and directly interacts with the homotypic fusion and protein sorting complex to mediate the fusion of endosomes and autophagosomes with lysosomes.

The lncRNA cardiac hypertrophy-associated transcript (Chast) can suppress autophagy via downregulating Pleckhm1 and possibly ATG5 expression to induce cardiomyocyte hypertrophy (Figure 5). This study sheds light on the mechanism of Chast/Pleckhm1 interaction during

![Figure 4: Brief summarization of the autophagosome elongation/closure step and its regulation by lncRNAs.](image-url)
autophagy flux to facilitate tumor proliferation, whereas associated ribonucleoprotein complexes.\textsuperscript{126} autophagy could also affect the expression of lncRNAs. If lncRNAs regulate autophagy, it would be interesting to know whether as an increasing number of lncRNAs have been identified to diabetes, and autophagy repression decreases its transcription and that this function can be interpreted through their association with autophagy. In consideration of the intimate linkage between lncRNAs and autophagy, it would be possible to develop lncRNA-based approaches to monitor or interfere with autophagy flux. As lncRNA expression is prevalent along the developmental and spatial axis, and several autophagy-associated lncRNAs described in this review virtually exhibit tissue specificity, such as HULC and PCA3, lncRNAs may serve as biomarkers of specific diseases, and pertinent therapeutic measures may be developed.\textsuperscript{103,133} Finally, both lncRNAs and autophagy are involved in a vast range of diseases including cancer; therefore, a joint intervention targeting lncRNAs and autophagy may be a promising therapeutic method.

Conflict of Interest
The authors declare no conflict of interest.

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Figure 5  Brief summarization of the autolysosome fusion and its regulation by lncRNAs. Fusion is the final step that autophagosome fuses with endosome and lysosome to form autolysosome, where the autophagic cargo is degraded. The lncRNA chast and MALAT1 have been found to regulate the step autolysosome fusion and implies that Chast is a possible target for the prevention of cardiac remodeling.

Additional lncRNA Regulators of Autophagy
Some lncRNAs, including lncRNA 7SL,\textsuperscript{121} BANCR,\textsuperscript{122} PCA3,\textsuperscript{123} LINC01116\textsuperscript{124} and CTA,\textsuperscript{125} also have been found to be related to autophagy, although the evidence of their correlation with ATG genes is lacking. BANCR\textsuperscript{122} can provoke autophagy flux to facilitate tumor proliferation, whereas 7SL,\textsuperscript{121} PCA3,\textsuperscript{123} LINC01116\textsuperscript{124} and CTA\textsuperscript{125} have the opposite effect.

lncRNAs Regulated by Autophagy
As an increasing number of lncRNAs have been identified to regulate autophagy, it would be interesting to know whether autophagy could also affect the expression of lncRNAs. Autophagy can degrade several types of RNAs and associated ribonucleoprotein complexes.\textsuperscript{126} Plasmodium variant translocation 1 (PVT1) is the sole lncRNA reported to be regulated by autophagy so far. PVT1 is upregulated in diabetes, and autophagy repression decreases its transcriptional level.\textsuperscript{127} The elevation of PVT1 mediated by autophagy functions crucially in the development and progression of diabetic nephropathy.\textsuperscript{128,129} Clearly, PVT1 is probably not degraded by autophagy, as it is downregulated when autophagy is repressed.\textsuperscript{127} Thus, extensive further investigations are needed to demonstrate what determinants participate in this process.

Conclusions
Given the limitations of the research that has been conducted to date, we have gained limited knowledge of the underlying mechanisms of regulation between identified lncRNAs and autophagy. The majority of lncRNAs typically function as sponges to sequester autophagy-related miRNAs from their targets.\textsuperscript{130,131} Certainly, lncRNAs have more complicated functions in autophagy regulation that are waiting to be elucidated, including but not limited to chromatin and histone remodeling, transcriptional regulation and protein–protein interactions.\textsuperscript{132} Current studies also indicate that we may need to classify lncRNAs according to their roles in distinct types of autophagy, such as mitophagy, to probe their function more specifically.\textsuperscript{47,89} From the growing knowledge based on lncRNAs and autophagy, we have formed the impression that most lncRNAs involved in autophagy have parts in tumorigenesis and that this function can be interpreted through their association with autophagy. In consideration of the intimate linkage between lncRNAs and autophagy, it would be possible to develop lncRNA-based approaches to monitor or interfere with autophagy flux. As lncRNA expression is prevalent along the developmental and spatial axis, and several autophagy-associated lncRNAs described in this review virtually exhibit tissue specificity, such as HULC and PCA3, lncRNAs may serve as biomarkers of specific diseases, and pertinent therapeutic measures may be developed.\textsuperscript{103,133} Finally, both lncRNAs and autophagy are involved in a vast range of diseases including cancer; therefore, a joint intervention targeting lncRNAs and autophagy may be a promising therapeutic method.
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