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MicroRNAs in autophagy and their emerging roles in crosstalk with apoptosis

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Abbreviations: BECN1, Beclin 1; BCL2, B-cell CLL/lymphoma 2; MCL1, myeloid cell leukemia sequence 1; BCL2L1, BCL2-like 1; VPS34, class III phosphatidylinositol 3-kinase; AMBRA1, autophagy/Beclin 1 regulator 1; RAB5A, RAS-associated protein RAB5A; LC3, microtubule-associated protein 1 light chain 3 alpha; ATG, AuTophaGy-related; tATG5, truncated form of autophagy-related protein 5; IRGM, immunity-related GTPase family, M; SQSTM1, p62/sequestosome 1; HATs, histone acetyltransferases; TRAIL, TNF- related apoptosis-inducing ligand; BAK1, BCL2-antagonist/killer 1; HDACs, histone deacetylases; BAX, BCL2-associated X protein; FADD, Fas-associated via death domain; DISC, death-inducing signaling complex; CASP, apoptosis-related cysteine peptidase; C-BECN1, C terminal fragments of BECN1; 3'UTR, 3' untranslated region

Macroautophagy (hereafter referred to as autophagy) is an evolutionarily conserved self-degradative process, which involves the regular turnover of cellular components via sequestering damaged macromolecules and transporting them for lysosomal degradation. In the past few years, the scientific community has produced remarkable advances in our understanding of the genes that are involved in autophagy and of their profound effects on various diseases. Recently, a new class of noncoding RNAs, known as microRNAs (miRNAs), has been demonstrated to play crucial roles in diverse biological processes including development, cell differentiation and apoptosis. Here, we review the current understanding about miRNAs focusing on their involvement in the autophagy process. Intriguingly, several confirmed targets of these autophagy-miRNAs are also important regulators in the crosstalk between autophagy and apoptosis. Furthermore, transcripts involved in autophagy and apoptosis may indirectly modulate each other by competing for common miRNA binding sites. Thus, miRNAs potentially work as molecular switches between these two intimately connected processes and contribute to the cell fate decision.

Introduction

Macroautophagy is an evolutionarily conserved cellular catabolic process in which proteins and organelles are eliminated through delivery to lysosomes.[1] Although in a few cases autophagy may play a role in the execution of cell death, it is usually a cytoprotective mechanism in maintaining homeostasis and protecting cells from nutrient stress.[2] Deregulation in autophagy has been implicated in numerous human disease, including developmental disorders, neurodegenerative disease and cancers.[1] Autophagy is an intrinsic cellular process, which needs to be tightly controlled. In addition, the autophagic pathway should actively exchange information with other cellular processes such as apoptosis. Recently, one group of endogenous noncoding RNAs, miRNAs, have been found to be involved in the regulation of autophagy, and their roles in modulating the crosstalk between autophagy and apoptosis are emerging.

miRNAs are ~22-nucleotide long regulatory molecules, which are usually phylogenetically conserved.[3] The biogenesis and action mechanism of metazoan miRNAs are summarized in **Figure 1**. miRNAs reside in protein-coding, intronic or intergenic regions throughout the genome. They can be produced from their own promoters or transcribed together with their host genes (in the cases of miRNAs located in introns). Mammalian miRNAs tend to cluster along the genome, and this clustering property plays an important role in guaranteeing the coordinate expression of different miRNAs.[4,5] Metazoan miRNAs are mainly transcribed by RNA polymerase II, which produce hundreds or thousands of nucleotide-long products called primary miRNAs (pri-miRNAs).[6] In the nucleus, the DROSHA nuclease complex cleaves metazoan pri-miRNAs into 70-nucleotide hairpins, known as precursor-miRNAs (pre-miRNAs). After being transported to the cytosol by XPO5 (exportin 5), pre-miRNAs are further processed into mature miRNAs by another RNase III DICER1.[3] The metazoan pre-miRNAs can also be produced from spliced introns. This "miRtron" pathway is conserved among diverse mammals as well as in drosophilids and nematodes.[7-9]

It had been thought that metazoan miRNAs lead to translation attenuation by targeting the 3'UTRs; however, novel findings challenged this notion because miRNA-mediated metazoan mRNA cleavage could also be observed in many cases.[10,11] The first 2–8 bases of a mature miRNA sequence usually play a pivotal role in target recognition in metazoans and are routinely used in most bioinformatics algorithms to search for target mRNAs.[12] Therefore, different miRNAs can share common target mRNAs if they possess similar "seed" regions.

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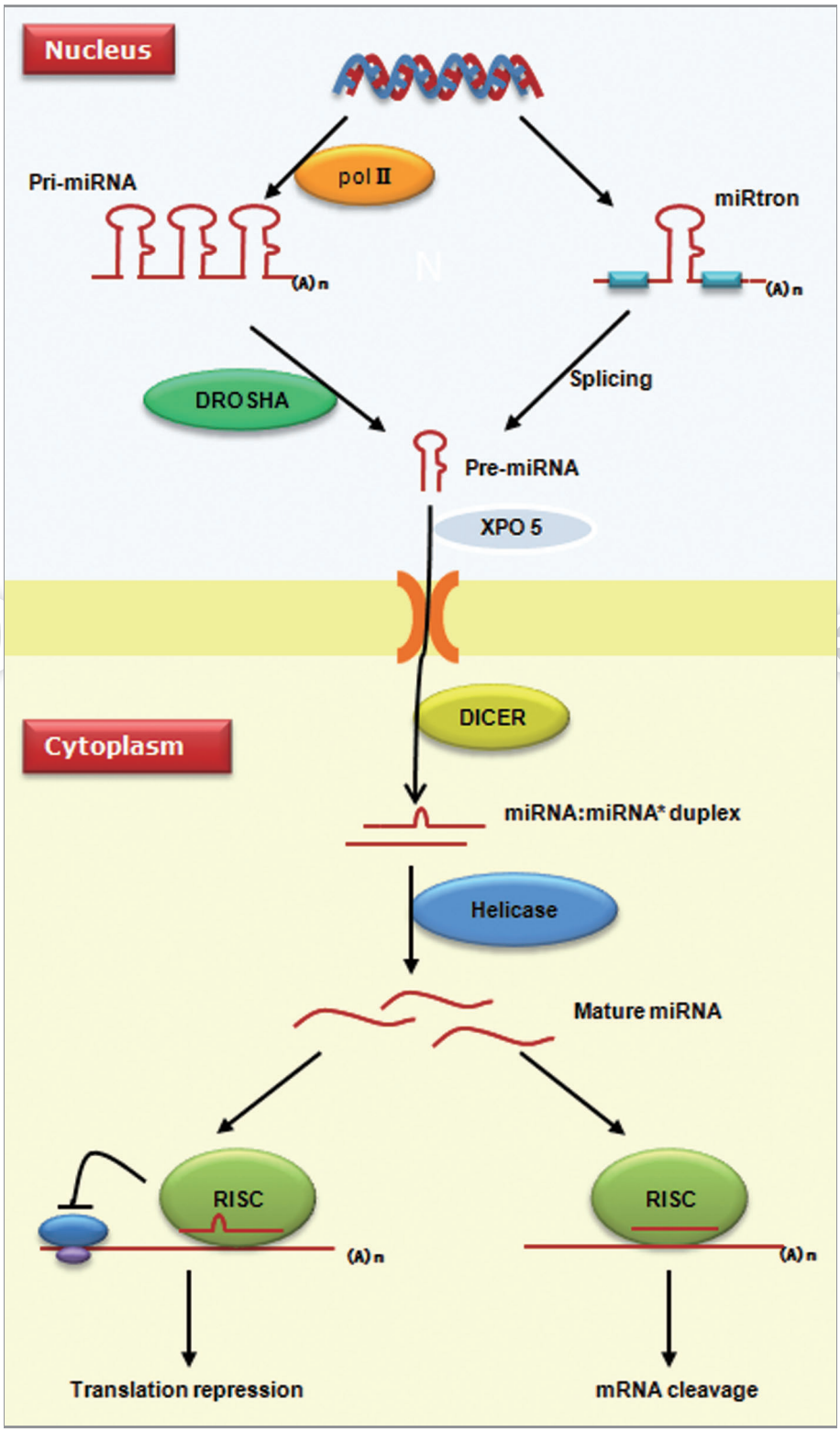

Figure 1. Biogenesis and action mechanism of miRNAs. Metazoan miRNAs are transcribed into pri-miRNA by RNA polymerase II. In the nucleus, precursor-miRNA can be produced either by processing of pri-miRNAs via the DROSHA complex or by the miRtron pathway without RNase. Pre-miRNAs are translocated into the cytoplasm by XPO5, where another RNase, DICER1, cleaves pre-miRNAs to generate a miRNA:miRNA* duplex. Single strands of mature miRNA enter into the miRISC complex to recognize target genes. miRNAs can silence a target gene via either repressing protein translation or enhancing mRNA degradation.

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miRNAs play a critical role in a broad range of biological processes such as developmental timing, differentiation and tissue morphogenesis.[3,13] Deregulation of miRNAs is also involved in a wide spectrum of diseases. For example, more than half of miRNA genes are located at fragile sites or cancer-associated genomic regions and are often aberrantly expressed in human cancers.[14,15]

miRNAs Regulate Autophagy and Contribute to Disease Progression

The regulatory role of miRNAs in autophagy was first uncovered in 2009 when *BECN1*, an important autophagy-promoting gene, was shown to be post-transcriptionally modulated by *MIR30A*.[16] Soon after this report, a number of miRNAs participating in autophagy have been associated with certain diseases including cancers, cardiac pathologies and Crohn disease. In the following, we will discuss these miRNAs and their relevance to autophagy (summarized in **Table 1** and **Fig. 2**).

MIR30A. The *MIR30* subfamily contains five paralogs: *MIR30A, B, C, D* and *E*, which map at different genomic positions. *MIR30A* and other members of the *MIR30* family are abundantly expressed in the adult prefrontal cortex.[17] Previous studies have found that *MIR30A* can bind to a conserved site at the 3'UTR of *BDNF* (brain-derived neurotrophic factor), a key regulator during cortical development and maturation.[17] Alterations in *BDNF* expression have been reported in a plethora of neuropsychiatric diseases.[18] A recent investigation suggested *MIR30* family members are also upregulated during cellular senescence. The *MYBL2* (*B-Myb*) oncogene, an important regulator of the cell cycle, was identified as a bona fide target of *MIR30A* during senescence.[19] Furthermore, blocking the activity of *MIR30A* inhibits cellular senescence.[19] All of the above lines of evidence indicate *MIR30A* is a potential tumor suppressor.

Recently, Zhu et al. demonstrated that *MIR30A* expression is inhibited when cells are subjected to nutrient depletion or rapamycin treatment, respectively.[16] *MIR30A* negatively regulates *BECN1* both at the mRNA and protein level in human breast, lung and glioma cancer cell lines. Overexpression of *MIR30A* leads to the *BECN1*-dependent suppression of autophagic activity in cancer cells. BECN1 is identified as a potent inducer of autophagy and plays a key role in tumorigenesis and neurodegenerative diseases.[20,21] Previous studies have documented the decreased expression of *BECN1* in human breast, ovarian and brain cancers.[20,22] This finding elucidated a novel regulation mechanism for *BECN1* and further suggested that blocking of *BECN1* by *MIR30A* may contribute to cancer progression.

***MIR206* and *miR-9-3p*.** *MIR206* is transcribed together with *MIR133B* as a miRNA cluster. Expression of *MIR133B* and *MIR206* increase during muscle cell differentiation and human fetus development.[23,24] A master myogenic transcriptional factor, *MYOD1*, induces *MIR206* transcription in muscle.[25]*MIR206* can block human rhabdomyosarcoma growth by targeting the *c-Met* 3'UTR both in rhabdomyosarcoma cell lines and in xenotransplanted mice.[23,24] In addition, *MIR206* is reported to markedly decrease in estrogen receptor α-positive human breast cancer tissues.[26] In MCF-7 breast cancer cells, overexpression of *MIR206* results in reduced cell proliferation and enhanced apoptosis via downregulating endogenous estrogen receptor α and other estrogen receptor-associated coregulatory proteins.[27,28] The human *MIR9* subfamily contains three genes, *MIR9-1*, *MIR9-2* and *MIR9-3*, which are separately located at 1q22, 5q14.3 and 15q26.1. *miR-9-3p* and *miR-9-5p* denote mature miRNAs originating from 3' and 5' arms of the same hairpin structure, respectively. *miR-9-3p* is significantly downregulated in the prefrontal cortex of subjects with schizophrenia compared with healthy controls.[29] Recently, it has been demonstrated that *MIR9* exerts its tumor suppressor role in various cancers including ovarian tumor,[30] gastric cancer,[31] cervical cancer[32] and breast cancer.[33] Expression of *MIR9* is activated by *MYC* (v-myc myelocytomatosis viral oncogene homolog) and *MYCN* (v-myc myelocytomatosis viral related oncogene, neuroblastoma derived) in breast cancer cells, which further contributes to metastasis.[33]

A recent investigation indicated increased expression of *MIR206* and reduced expression of *miR-9-3p* in primary

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Table 1. miRNAs with relevance in autophagy

miRNAs	Chromosome location	Disease relevance	Targets relevant to autophagy	Function	References
MIR30A	6q13	Targets B-Myb oncogene during cellular senescence	*BECN1*	Regulation of autophagic response to rapamycin in cancer cells	16, 19
MIR206	6p12.2	Blocks the growth of human rhabdomyosarcoma and breast cancer cells	*KAT6A (MYST3)*	Modulation of histone acetylation	23, 27, 28, 35
miR-9-3p	1q22, 5q14.3, 15q26.1	Downregulated in the prefrontal cortex of schizophrenic subjects; contributes to tumor metastasis	*HDAC4* and *HDAC5*	Modulation of histone acetylation	29, 33, 35
MIR101	1p31.3, 9p24.1	Locus is lost in clinically localized prostate cancers or metastatic prostate cancers	*RAB5A, ATG4D* and *STMN1*	Sensitizes breast cancer cells to 4-OHT-mediated cell death.	43, 45
MIR17,20,93 and 106	13q31.3, 7q22	Elevated in B-cell lymphoma and modulate tumor formation	*SQSTM1 (p62)*	Promote hematopoietic cell expansion	56, 58
MIR204	9q21.12	Downregulated in the NCI60 tumor cell lines; Involved in pulmonary arterial hypertension	*MAP1LC3*	Regulation of cardiomyocyte autophagy induced by hypoxia-reoxygenation	62–64
MIR196	17q21.32, 12q13.13, 7p15.2	Potential roles in melanoma and acute leukemia	*IRGM*	Deregulation of IRGM-dependent xenophagy	67, 68, 73

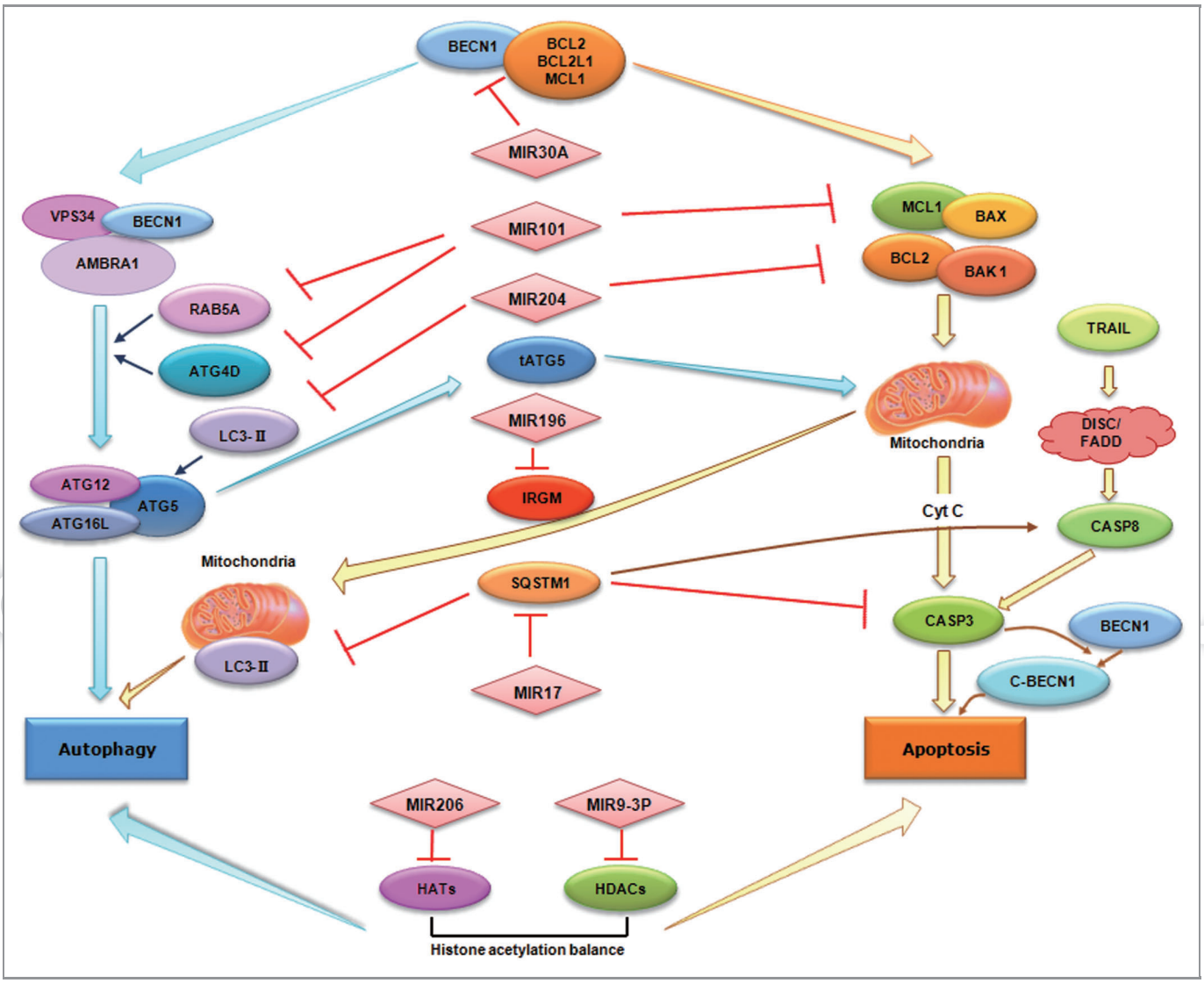

Figure 2. miRNAs in autophagy and their emerging roles in crosstalk with apoptosis. miRNAs can directly target autophagy-associated proteins such as BECN1 and RAB5A. Alternatively, miRNAs may indirectly modulate autophagy regulators such as HDACs and HATs. As shown in the figure, miRNAs can also mediate the crosstalk between autophagy and apoptosis via either targeting the common regulators for both pathways such as *BECN1* and *SQSTM1*, or via regulating multiple targets involved in the two pathways.

waldenstrom macroglobulinemia (WM) cells.[34] WM is a rare, nonHodgkin lymphoma characterized by an arrest of B lymphocytes after somatic hypermutation and before isotype class switching. The abnormal expression of these two miRNAs leads to alteration of balances between autophagy and apoptosis via modulating histone acetylation in WM cells.[35]

Histone deacetylases (HDACs) and histone acetyl transferases (HATs) control the chromatin structure status, and the balance of these two families of enzymes is crucial to gene transcription.[36] In many malignancies including WM, this balance is disrupted, which is characterized by significantly increased expression of HDACs and by significantly decreased expression of HATs.[34] Since *HDAC4*, *HDAC5* and *KAT6A* (*MYST3*) were identified as targets for *miR-9-3p* and *MIR206*, respectively, the increased expression of *MIR206* and reduced expression of *miR-9-3p* deregulate histone acetylation and lead to autophagy-dependent cell toxicity.[35] It should be noted that although the modulation of autophagy by HDAC suppression has been implicated in the pathogenesis of human diseases,[37,38] how histone acetylation affects autophagy remains largely unknown. For example, Shao et al. found that suberoylanilide hydroxamic acid, a potent inhibitor of HDAC, can induce cancer cell death, which had unambiguous morphological features of autophagosome formation.[37] However, Cao et al. recently reported that suppressing of HDAC attenuates cardiac hypertrophy via autophagy inhibition.[38] How can this discrepancy be explained? An obvious explanation is cell-type specificity. Alternatively, cells may choose different routes to link HDAC with autophagy. Therefore, further investigations are needed to delineate the underlying molecular mechanism.

MIR101. *MIR101* is an established tumor suppressor across many cancer types.[39-44] It has two genomic loci, which are on chromosome 1 (*MIR101-1*) and chromosome 9 (*MIR101-2*). Based on genomic PCR, one or both of the two genomic loci are found to be somatically lost in clinically localized prostate cancers or metastatic prostate cancers.[43] *EZH2* (Enhancer of zeste homolog 2), which encodes a mammalian histone methyltransferase that epigenetically regulates cancer cell survival and metastasis, was identified as a key target of *MIR101* in prostate cancer cells and in non-small cell lung cancers.[43,44] Other confirmed targets for *MIR101* include *MCL1* (myeloid cell leukemia sequence 1) and *FOS* (FBJ murine osteosarcoma viral oncogene homolog) oncogene in hepatocellular carcinoma,[40,42] *MAGI2* (membrane-associated guanylate kinase, WW and PDZ domain containing 2) in breast cancer cell lines[41] and the *MYCN* gene in neuroblastoma cell lines.[39] Therefore, *MIR101* promotes apoptosis and suppresses tumorigenesis.

Interestingly, in a functional screen for miRNAs that regulate the autophagic flux in breast cancer cells, *MIR101* was found to be a potent inhibitor of autophagy.[45] *MIR101* targets the genes encoding the autophagy-related proteins *RAB5A*, *ATG4D* and *STMN1*. *RAB5A* is a member of the *RAS* oncogene family, which is conserved from yeast to humans. The *RAB5A* protein can exhibit GTPase activities and is identified as a key regulator of endocytosis.[46] However, a recent investigation suggested *RAB5* is involved in autophagosome formation and regulates *ATG5–ATG12* conjugation.[47] In addition, siRNA inhibition of *RAB5A* blocks basal and rapamycin-induced autophagy.[45]

ATG4D belongs to the ATG4 family of cysteine-type endopeptidases, which is a homolog of yeast Atg4.[48] In mammals, ATG4 cleaves the C terminus of LC3 (yeast Atg8) to form cytosolic LC3-I, which is covalently conjugated to the lipid phosphatidylethanolamine (PE) on autophagosomal membranes. Atg4-Atg8 conjunction is a crucial step in the autophagosome biogenesis pathway. Currently, four mammalian *ATG4* paralogs [*ATG4A-ATG4D*] and six *ATG8* paralogs with varied substrate specificity have been cloned. Previous studies suggested that although ATG4B is the main regulator of LC3 in mammalian cells, other members of the ATG4 family may be specific for other individual Atg8 orthologs.[48,49]

STMN1 is a gene coding for a ubiquitous cytosolic phosphoprotein. Previous studies suggested STMN1 modulates depolymerization of interphase and mitotic microtubules based on the transition of unphosphorylated and phosphorylated forms.[50] *STMN1* is ectopically expressed in a variety of cancer types.[51] Silencing of *STMN1* inhibits tumor growth in breast cancer cells, primary melanomas and osteosarcomas.[52,53] Excessive expression of *STMN1* causes a partial block of *MIR101*-mediated inhibition of autophagy, indicating its importance as a *MIR101* target.[45]

***MIR17*, *20*, *93* and *106*.** The *MIR17*, *20*, *93* and *106* genes, are highly conserved between species and share the same AAGUGC 'seed' region and target specificity. *MIR17* and *MIR20* belong to the *MIR17-92* cluster. The human *MIR17-92* cluster is mapped to 13q31.3, a region amplified in diffuse B-cell lymphomas (DLBCLs), follicular lymphomas, Burkitt's lymphomas and lung carcinoma.[54] The *MIR17-92* cluster is markedly elevated in B-cell lymphoma and lung cancers.[55,56] *MIR106B*, *MIR205* and *MIR93* consist of a paralog of the *MIR17-92* cluster. This miRNA cluster locates at chromosome 7q22, a region also amplified in several cancers. In recent years, the oncogenic properties of the *MIR17-92* and *MIR106-25* clusters have been extensively investigated.[55,56] These two clusters are induced via MYC and E2F1 signals. Subsequent overexpression of these miRNAs downregulates p21 which is required for cell cycle arrest.[57] *MIR106A*, located in the *MIR106A-92* cluster, is the second paralog of *MIR17-92*. Although the function of this cluster remains obscure, it may also be associated with oncogenic development at least in cancer cells.

Recently, these AAAGUGC seed-containing miRNAs were found highly expressed in myeloid progenitors and blocked in mature neutrophils.[58] SQSTM1 (sequestosome 1/p62), a multiple domain protein that acts as a signaling hub, was identified as a key target for these miRNAs. SQSTM1 can interfere with autophagy via binding to the autophagic regulator Atg8/LC3.[59] SQSTM1 has also been implicated in a variety of cellular events such as the NFκB and proteasome pathways.[60] During ligand-induced neutrophil differentiation, SQSTM1 regulates colony-stimulating factor 3 receptor stability and mitogen-activated protein kinase signaling. Therefore, these AAAGUGC seed-containing miRNAs enhance the expansion of myeloid 32D cells and primary hematopoietic progenitors by modulating SQSTM1-regulated cellular events.[58] Notably, in autophagy-defective and apoptosis-incompetent tumor cells, metabolic stress results in SQSTM1 aggregation, which further triggers a positive feedback loop for the generation of reactive oxygen species, enhanced genomic instability and tumorigenesis.[61] Thus, elimination of SQSTM1 through upregulation of AAAGUGC seed-containing miRNAs may potentially inhibit the proliferation of these tumor cells.

MIR204. *MIR204* is located within the intron of the *TRPM3* gene at 9q21.12. Its expression is downregulated in pulmonary artery smooth muscle cells and in clinical samples, suggesting that *MIR204* plays a critical role in the etiology of human pulmonary arterial hypertension.[62] In addition, compared with normal tissues, *MIR204* is significantly lower in the NCI60 tumor cell line panel.[63] In a recent study, Xiao et al. found that hypoxia-reoxygenation induces cellular autophagy in cardiomyocytes of neonatal rats.[64] Concurrently, *MIR204* is significantly decreased and the ratio of *LC3-II/LC3-I* is increased. However, it remains to be determined whether *MIR204* inhibits autophagy by directly targeting *MAP1LC3*.

MIR196. *MIR196* is an evolutionarily conserved miRNA in vertebrate species. This family consists of three members including two *MIR196A* genes (*MIR196A1* and *MIR196A2*) and one *MIR196B* gene. These miRNAs appear to be expressed from intergenic regions in *HOX* gene clusters and mediate the posttranscriptional restriction of *HOX* gene expression during development.[65,66] Recent studies have extended the function of *MIR196* to tumorgenesis. For example, *MIR196* can repress several transcription factors and play a regulatory role in melanoma and acute leukemia.[67,68] Indeed, a number of genetic polymorphisms in the precursor or mature *MIR196* have been

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associated with susceptibility and risk in different cancer types.[69-71]

Crohn disease is a complex inflammatory bowel disease, which can affect any area of the gastrointestinal tract, from the mouth to the anus.[72] *MIR196* is overexpressed in the inflammatory intestinal epithelia of individuals with Crohn disease.[73] Bioinformatics analysis revealed the potential recognition sequence of *MIR196* on the coding region of the *IRGM* (immunity-related GTPase family, M gene). Interestingly, a synonymous variant polymorphism of *IRGM* is located within the 'seed' region. Subsequent functional analysis indicated that *MIR196* downregulates the *IRGM* protective variant (c.313B) but not the risk-associated allele (c.313T).[73] The involvement of *IRGM* in the innate immune response is mediated via regulating autophagy in response to intracellular pathogens, or xenophagy through mitochondria.[74] Therefore this is the first example suggesting a miRNA-associated synonymous polymorphism influencing autophagy and disease risk.

Roles of miRNAs in Crosstalk between Autophagy and Apoptosis

Apoptosis is a controlled process of cell death occurring when cells face irreversible stress. By apoptosis, cells eliminate the damaged cells that may be harmful to the organism and maintain normal cell homeostasis. Apoptosis can be induced either by various cellular insults mediated through the mitochondria (intrinsic pathway) or cell surface death receptors (extrinsic pathway). Members of the BCL2 family play crucial roles in regulating apoptosis.[75] Upon induction, upstream BH3-only proteins (such as BCL2L11/BIM, BAD and BBC3/PUMA) inactive anti-apoptosis BCL2 family members (BCL2, MCL1, BCL2L1). Then these anti-apoptotic family members relieve pro-apoptotic BAX and BAK1, which are translocated to the mitochondrial membrane and result in cytochrome c release and mitochondrial fission. In the extrinsic apoptosis pathway, components of the death-inducing signaling complex (DISC) including surface receptors of the death receptor, adaptor proteins (FADD and TRADD) and CASP8 and CASP10 are activated upon stimuli. Both intrinsic and extrinsic apoptotic pathways converge on the level of CASP3 activation, which in turn cleaves various intracellular substrates and cause the morphological changes observed in apoptotic cells.[75]

Both autophagy and apoptosis play important roles in the development, cellular homeostasis and oncogenesis of mammals. They may be triggered by common upstream signals, resulting in combined autophagy and apoptosis, or be mutually exclusive.[76,77] Intriguingly, several confirmed targets of autophagy-miRNAs are also important mediators in the crossregulation between autophagy and apoptosis (**Fig. 2**).

miRNAs can inhibit the common regulators of these two pathways. For example, previous investigations have suggested the physical interaction between BECN1 and proteins in the anti-apoptotic family (BCL2, MCL1, BCL2L1) is pivotal for the conversation between the two pathways.[78-80] Under normal conditions, BECN1 and anti-apoptotic BCL2 family members can bind to each other to maintain cellular homeostasis. When cells face stress conditions, BECN1 and BCL2 family members disassociate, thereby promoting autophagy and inhibiting apoptosis, respectively.[78-80] In addition to *MIR30A*, which can reduce the cytoplasmic level of BECN1, several other miRNAs have been demonstrated to reduce the expression level of the anti-apoptotic family members such as BCL2 and MCL1.[81,82] Thus, miRNAs may be actively involved in the regulation of both autophagy and apoptosis signals based on modulation of the protein-protein interactions. SQSTM1 is another common mediator under the control of miRNAs. Recent data suggest SQSTM1 can modulate the polyubiquitination and aggregation of CASP8, which is essential for the extrinsic apoptotic pathway.[83] On the other hand, SQSTM1 can negatively regulate the degradation of the autophagic protein LC3 by the 20S proteasome.[84] Therefore, collective evidence implicates a potential mechanism of SQSTM1 underlying the interplay between the apoptosis and autophagic pathways.

In fact, autophagy and apoptosis share many essential genes, ranging from common players such as *TP53* (*p53*) and *ATG5* to signal transduction mediators such as DAPK1 (death-associated protein kinase 1) and EEF2 (eukaryotic translation elongation factor 2).[76,85,86] It would be interesting to identify whether or not other miRNAs could coordinately regulate both apoptosis and autophagy signals based on modulating these proteins. Alternatively, as we summarized in **Figure 2**, some miRNAs can simultaneously modulate multiple targets, which function either in autophagy or in apoptosis. For example, *MIR101* potently targets the genes encoding the autophagy-associated proteins RAB5A, ATG4D and STMN1; and also the anti-apoptotic protein MCL1.[42,45] During hypoxia-reoxygenation, *MIR204* blocks autophagy by modulating the LC3-II protein whereas in cholangiocarcinoma cells the exogenous expression of *MIR204* negatively regulates BCL2 and facilitate chemotherapeutic drug-triggered apoptosis.[87]

Working Models for the Mechanisms of miRNAs in Autophagy and the Crosstalk with Apoptosis

Previous proteolysis analyses have suggested most miRNAs only downregulate their individual target moderately.[88,89] However, some miRNAs can function as molecular on-off switches to completely shut down a cellular process. The trick perhaps lies in the multitarget characteristics of miRNAs. Since one single miRNA can concurrently target multiple genes, the multiple autophagy-related proteins that control different steps of autophagy may be regulated by the same miRNAs (**Fig. 3A**). Indeed, in the above *MIR101* example, this miRNA represses three important autophagy-associated genes. Importantly, overexpression of *STMN1* can only partially rescue *MIR101*-mediated autophagic inhibition.[45] This result indicates that other *MIR101* targets including *RAB5A* and *ATG4D* still act in autophagy, and further strengthens the point that a single miRNA can take many routes to modulate autophagy.

Alternatively, several coregulated miRNAs can modulate the same or different autophagic steps in a cooperative manner,

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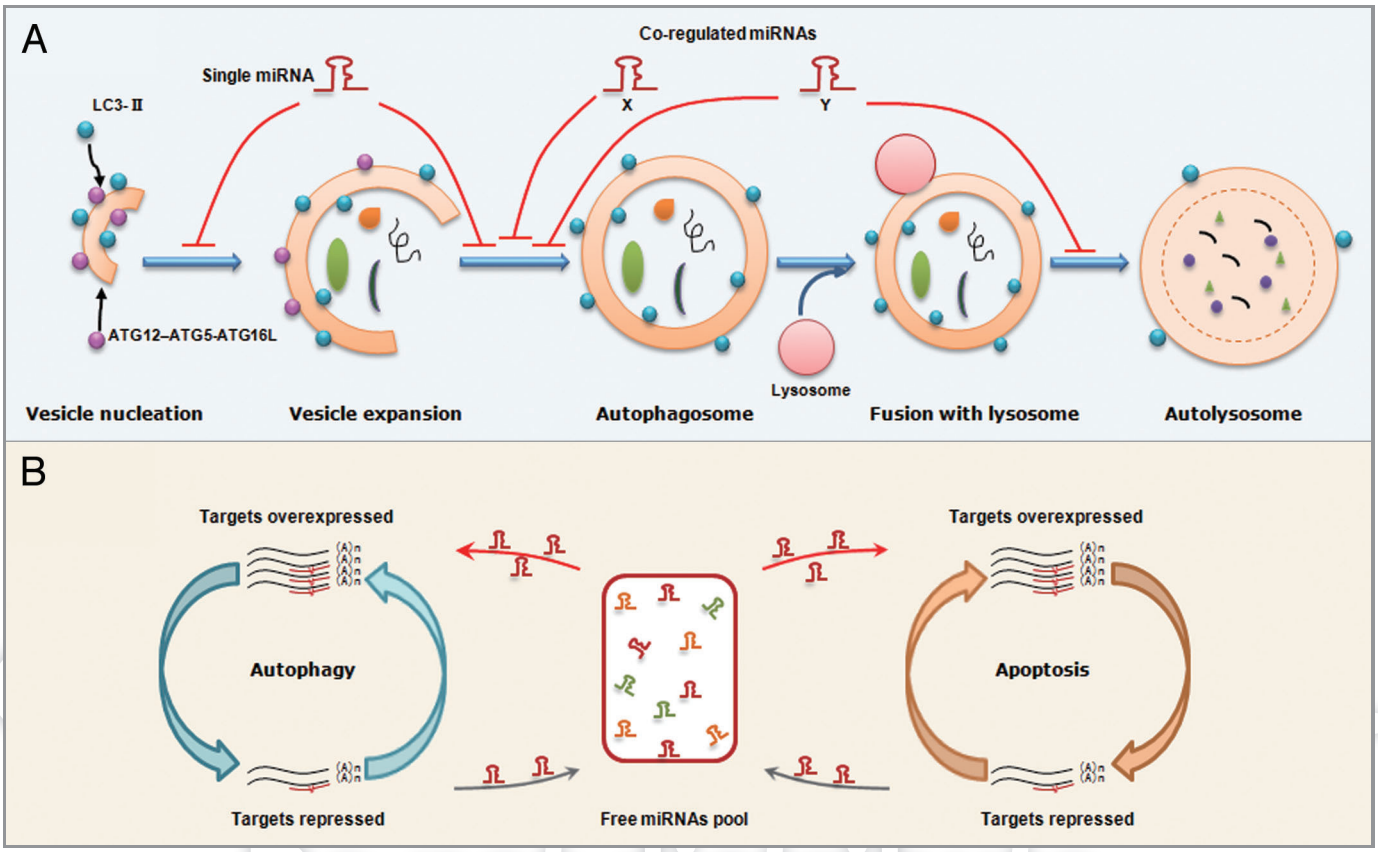

Figure 3. Proposed models that miRNAs regulate the autophagy and the balance between autophagy and apoptosis. (A) An illustration of miRNA regulation of autophagy. A single miRNA can modulate multiple targets in different steps of autophagy. Alternatively, several co-regulated miRNAs can modulate the different steps in a cooperative manner although each miRNA regulates its specific target. (B) A working model for miRNA-mediated crosstalk between autophagy and apoptosis. Transcripts involved in different cellular processes but with common miRNA binding sites can modulate each other by competing for miRNA binding. For example, overexpression of autophagy-related genes will result in binding of more miRNA molecules, thereby leading to fewer miRNA molecules free to bind to apoptosis-related transcripts, which share similar miRNA binding sites. Thus, the miRNA-medicated conversation between autophagy and apoptosis is regular and intensive.

although each miRNA regulates its specific target (**Fig. 3A**). This situation is illustrated in the *MIR17* seed family example. *MIR17*, *20*, *93* and *106*, which all contain an AAAGUGC seed region, are expressed in hematopoietic cells at different stages of myeloid development.[58] Experiments demonstrate that they all regulate the same target, *SQSTM1*.[58] In fact, this cooperation may not be restricted to a set of miRNAs with a similar seed. For example, previously we and others independently found a functional link between members within the same miRNA cluster.[4,90] Since mammalian miRNAs with a proximal locus tend to share the same transcriptional unit, upon stimulation, different members of the same miRNA cluster can be concurrently induced or repressed, thus controlling a biological process in a cooperative manner.

Since autophagy and apoptosis share some essential mediators, the direct targeting of the common proteins by miRNAs will ultimately affect signal transduction in both pathways (**Fig. 2**). In addition, we speculate that transcripts involved in autophagy and apoptosis can indirectly modulate each other by competing for common miRNA binding sites. As indicated in the schema shown in **Figure 3B**, when autophagy-related proteins are repressed, more miRNA molecules are liberated into the free miRNA pool and further enter into the apoptotic pathway to target apoptosis-related proteins. By contrast, the accumulation of genes encoding autophagy-related proteins will result in the binding of more miRNA molecules, thereby leading to fewer miRNAs being free to bind apoptosis-related transcripts with similar binding sites. Thus, the miRNAs maintain regular and intensive crosstalk between autophagy and apoptosis.

This miRNA-mediated mutual regulation between transcripts with similar binding sites has great implications in cancer biology. Poliseno et al. found that *PTENP1*, the pseudogene of *PTEN*, possesses well-conserved miRNA binding sites and is biologically active since it can compete with *PTEN* for miRNA binding. Therefore *PTENP1* modulates the cellular levels of PTEN and exerts a tumor suppressor role.[91] Since this mechanism depends only on the competition between the 3'UTRs, it can be expected that it is not limited to this gene and its pseudogene. Indeed, the same group of researchers discovered that there are a network of competing endogenous mRNAs that in a mutually reciprocal manner center on *PTEN*.[92,93] Recently, a set of investigations has extend this concept to long-coding RNAs and to all

protein-coding mRNAs.[94,95] Based on the shared miRNA binding sites, protein-coding genes within the autophagic and apoptotic pathways can maintain cellular homeostasis and collectively decide cell fate. Deregulation of this miRNA-based "housekeeping" mechanism may contribute to disease progression.

Conclusions and Perspectives

Over the past few decades, tremendous interest has focused on understanding the regulatory role of miRNAs under various physiological and disease conditions. In this review, we summarized the recent findings concerning miRNAs involved in autophagy. Ever since the first autophagy-associated miRNA, *MIR30A*, was discovered in 2009, our knowledge of miRNAs in autophagy has accumulated rapidly. However, the understanding of this field is still in its infancy. For example, direct targeting of autophagy-related genes has only been experimentally shown in a few cases at present.[16,45,58,73] Since the identification of gene products that participate in autophagy is continually expanding,[96] it is likely that other autophagy-associated miRNAs will be found in the near future. Besides, it is important to develop efficient research tools for manipulating autophagy. We propose that miRNAs may be used to block autophagy-associated genes at both the mRNA and protein levels. As seen in the *MIR101* example, one single miRNA is enough to block both basal and rapamycin-induced autophagy via targeting multiple autophagy-associated genes.

miRNAs also represent an additional layer in the intricate interconnection between autophagy and apoptosis. As we discussed above, miRNAs may target the common regulators for both processes or simultaneously modulate multiple targets essential to each pathway. Recent developments have further indicated that miRNAs may work as a general mediator between protein-coding genes based on shared binding sites.[97] Therefore, multiple miRNAs together with their multiple downstream genes form a complicated regulation network. Use of systems level analysis, such as the efforts made in analyzing transcriptional and miRNA-based post-transcriptional regulation in the autophagy-lysosomal pathway, will help to elucidate the underlying connection across cellular processes.[98] In addition, a detailed deciphering of the crucial role of miRNAs in the interplay between autophagy and apoptosis have profound clinical implications since the evasion of cell death underlies tumorigenesis and represents a major obstacle to successful therapies.[99] Therefore, such efforts are imperative to improve our understanding of miRNAs in tumorigenesis and facilitate the design of appropriate therapies targeting this novel group of molecules.

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Note

After this manuscript was revised, the role of *MIR376B* on autophagy was reported in a recent paper.[100]

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