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MIR-1265 regulates cellular proliferation and apoptosis by targeting calcium binding protein 39 in gastric cancer and, thereby, impairing oncogenic autophagy

Zhipeng Xu[a,1], Zheng Li[a,1], Weizhi Wang[a,1], Yiwen Xia[a,1], Zhongyuan He[a,1], BoWen Li[a], Sen Wang[a], Xiaoxu Huang[a], Guangli Sun[a], Jianghao Xu[a], Lu Wang[a], Qiang Zhang[a], Qiang Li[a], Jialun Lv[a], Linjun Wang[a], Lu Zhang[a], Diancai Zhang[a], Hao Xu[a], Zekuan Xu[a,b,*]

[a] Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, 210029, Jiangsu province, China
[b] Collaborative Innovation Center For Cancer Personalized Medicine, Nanjing Medical University, Nanjing, 210029, Jiangsu province, China

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ABSTRACT

Increasing evidence indicates that microRNAs (miRNAs) play an important role in various tumors by regulating downstream target genes and diverse signaling pathways. Herein, we confirmed miR-1265 expression in gastric cancer (GC) using the Cancer Genome Atlas (TCGA) database and assessed the level of miR-1265 expression in clinical specimens and cell lines. We found that miR-1265 expression was negatively correlated with tumor size. Further functional analysis revealed that miR-1265 suppresses cellular proliferation and autophagy while inducing apoptosis in GC cells. A luciferase reporter assay was used to identify an miR-1265 targeted gene, calcium binding protein 39 (CAB39), which is an essential upstream regulator in the AMPK-mTOR signaling pathway. Upregulation or downregulation of CAB39 expression reversed the effects of miR-1265 overexpression or inhibition, respectively. Notably, the knockdown of autophagy-related gene 12 (ATG12) impaired the effects of miR-1265 inhibition or CAB39 overexpression in GC. MiR-1265 also suppressed the growth of GC cells in vivo and that of human gastric organoids. Altogether, our results show that miR-1265 suppresses GC progression and oncogenic autophagy by reducing CAB39 expression and regulating the AMPK-mTOR signaling pathway. Therefore, miR-1265 may represent a potential therapeutic target for GC.

1. Introduction

Gastric cancer (GC) is the fifth most common cancer worldwide, and it is ranked third among causes of death [1]. According to the statistics reported by Chen et al., 679,100 cases of GC were estimated to emerge in China in 2015, causing 498,000 deaths [2]. Although methods for the diagnosis and treatment of early GC have been developed, the long-term survival rate of patients with advanced GC remains low [3]. Therefore, it is clinically significant to clarify the mechanism underlying GC, which will help elucidate specific molecular targets and develop more effective therapies for this disease.

MicroRNAs (miRNAs) are a class of endogenous small noncoding RNA molecules that are approximately 19–22 nucleotides in length, and they regulate gene expression via binding to the 3′-UTR of mRNAs [4,5]. Over the past decade, the dysregulation of miRNAs has been associated with the biological activities of many tumors [6]. Moreover, several miRNAs have been shown to be involved in tumor development and might be applied as diagnostic or prognostic markers in GC [7]. It was reported that miR-1265 was involved in fibrosis in human renal proximal tubule cells, endometritis, and major depressive disorder [8–10]. A previous study has suggested that miR-1265 might be a potential marker for paclitaxel sensitivity in laryngeal cancer [11]. Herein, we confirmed that miR-1265 expression was significantly lower in GC specimens based on the Cancer Genome Atlas (TCGA) database. Because the biological functions of miR-1265 have seldom been reported, we concentrated on the role of miR-1265 in GC.

Autophagy is the major intracellular degradation system that delivers cytoplasmic materials to the lysosomes for degradation [12]. In addition, autophagy is involved in many normal cellular mechanisms, including inflammation, cell death, and immunity [13,14]. Moreover,

* Corresponding author. Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou road, Nanjing, 210029, Jiangsu province, China.
E-mail address: xuzekuan@njmu.edu.cn (Z. Xu).
[1] These authors contributed equally to this work.

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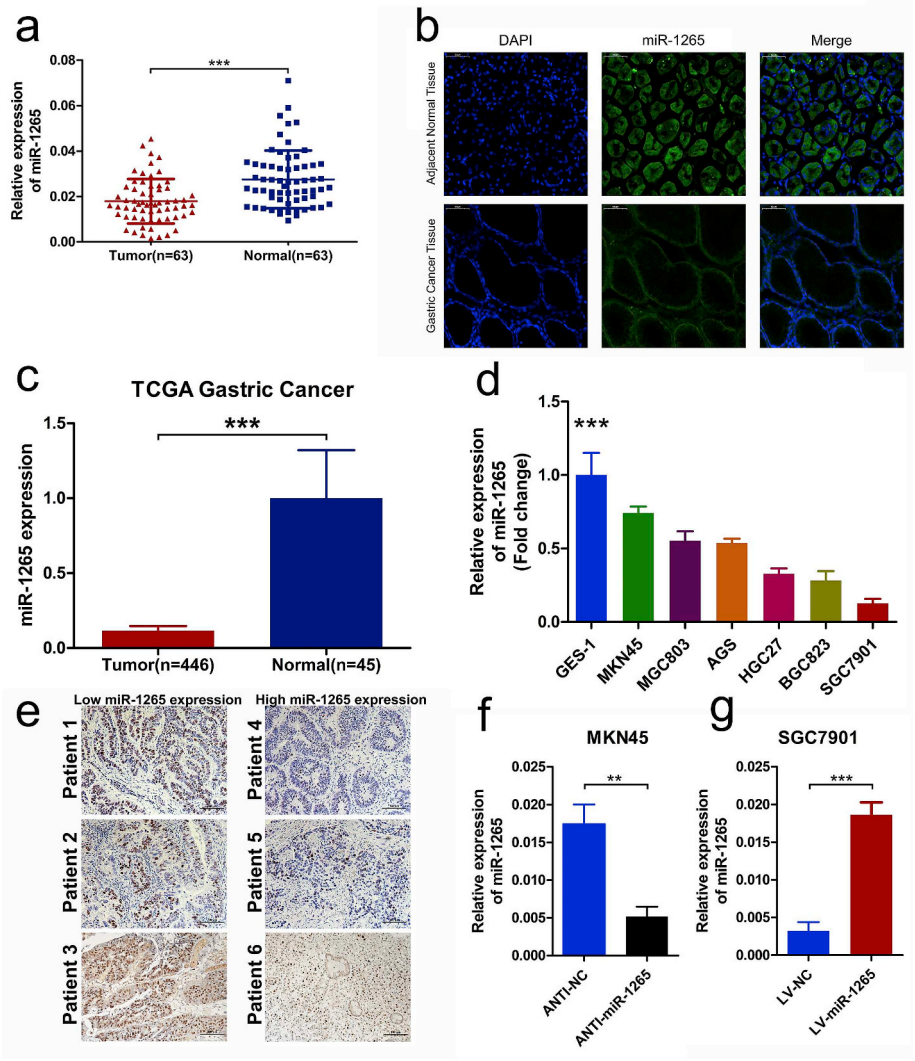

Fig. 1. MiR-1265 expression is downregulated in GC tissues and cell lines. (a) MiR-1265 expression in 63 pairs of GC specimens and paired adjacent gastric mucosa specimens using miRNA qRT-PCR. (b) Representative FISH images of miR-1265. (c) MiR-1265 expression in GC samples and paired normal samples in the TCGA database. (d) MiR-1265 expression in cell lines was evaluated using miRNA RT-PCR (one-way ANOVA and Dunnett's multiple comparison test). (e) Immunohistochemical staining of Ki-67 in GC tissues. (f,g) MiR-1265 expression in GC cell lines transfected with miR-1265 mimics or inhibitor lentivirus.

autophagy functions as a barrier to tumor initiation. After neoplastic lesions have been established, autophagy exerts a positive effect on cancer progression and maintenance [15,16]. During tumor progression, autophagy provides substrates that assist tumor cells in overcoming nutrient limitations and hypoxia [17,18]. Moreover, it has been shown that the inhibition of autophagy genes in tumor cells can induce cell death [19,20].

CAB39 (calcium-binding protein 39; MO25α) forms a heterotrimeric complex with an ste20-related adaptor and liver kinase B1 (LKB1); this complex acts as a major upstream regulator of AMP-activated protein kinase (AMPK) in mammalian cells and phosphorylates AMPK at Thr172 [21,22]. In mammalian cells, AMPK exhibits a number of functions, including the regulation of metabolism, cell polarity, and autophagy [22]. In some tumors, the loss of AMPK activity cooperates with oncogenic drivers, reprograming cellular metabolism and enhancing cellular proliferation [23]. On the other hand, AMPK activation can also play a promoting role in tumor progression by regulating cellular metabolism and, thus, allowing cells to adapt to metabolic stress [23–25]. Godlewski et al. demonstrated that the CAB39/LKB1/AMPK pathway can contribute to cellular adaptation to altered energy availability in glioma cells [26]. In addition, Jiang et al. reported that CAB39 promotes hepatocellular carcinoma growth and metastasis [27]. Similarly, Gu et al. found that autophagy mediated by the upregulation of LKB1/AMPK/mTOR signaling was used as a survival strategy in pancreatic cancer [28]. Despite CAB39 having been characterized as an oncogenic factor for glioma, hepatocellular carcinoma, and pancreatic cancer, the association between CAB39 and GC remains unknown.

Herein, we provide evidence of a significant decrease in miR-1265 expression in GC. Further experiments explored the functions of miR-1265 and its association with CAB39 in GC. In the present study, the upregulation of miR-1265 expression was found to influence the progression of GC by downregulating the expression of CAB39.

2. Materials and methods

All the materials and methods are included in Supplementary Materials and Methods.

3. Results

3.1. Downregulation of miR-1265 in GC clinical specimens and cell lines

To determine whether miR-1265 is dysregulated in GC specimens, we analyzed the expression of miR-1265 using RT-PCR in 63 pairs of GC

Table 1
Expression of miR-1265 and CAB39 in human gastric cancer and the clinicopathological characteristics of the patients.

Characteristics	Total (n = 63)	miR-1265 expression		P-value	CAB39 expression		P-value
		High group	Low group		High group	Low group	
Age(years)							
> 60	38	18	20	0.503	22	16	0.165
≤60	25	14	11		10	15	
Gender							
Male	47	23	24	0.613	20	27	0.051
Female	16	9	7		12	4	
Size(cm)							
< 3.5	30	20	10	0.016	11	19	0.032
≥3.5	33	12	21		21	12	
Histology grade							
Well-moderately	22	13	9	0.335	10	12	0.535
Poorly-signet	41	19	22		22	19	
Stage							
I/II	36	21	15	0.167	17	20	0.359
III	27	11	16		15	11	
T grade							
T1 + T2	26	14	12	0.685	11	15	0.259
T3 + T4	37	18	19		21	16	
Lymph node metastasis							
Present (N1-N3)	38	16	22	0.089	23	15	0.057
Absent(N0)	25	16	9		9	16	

*p < 0.05 Statistically significant difference.

specimens and matched adjacent normal specimens from patients with GC. MiR-1265 expression was lower in the tumor specimens than in the adjacent normal specimens (Fig. 1a). Next, we detected miR-1265 expression using fluorescence in situ hybridization (FISH) in three GC specimens and corresponding adjacent normal specimens, and we obtained consistent results (Fig. 1b). We then analyzed miR-1265 expression in the TCGA database and confirmed that miR-1265 expression was significantly decreased in the GC specimens (Fig. 1c). We also detected miR-1265 expression in cell lines using miRNA RT-PCR. MiR-1265 expression was substantially lower in the GC cell lines than in the normal gastric mucosa cell line GES-1 (Fig. 1d). In addition, the correlation between miR-1265 expression and the clinicopathological characteristics of patients with GC was analyzed (Table 1, Supplementary Table S1). We divided 63 patients with GC into two subgroups based on miR-1265 expression and found that miR-1265 expression was inversely correlated with tumor size. Furthermore, we selected tumor samples representative of each subgroup for Ki-67 staining. Compared with the tumors exhibiting low miR-1265 expression, tumors with high miR-1265 expression were associated with a lower proliferation index (Fig. 1e).

3.2. miR-1265 suppresses proliferation and induces apoptosis in GC cells

According to their level of miR-1265 expression, the MKN45 and SGC7901 GC cell lines were transfected with inhibitor lentivirus or miR-1265 mimics, respectively. MiRNA RT-PCR was utilized to assess miR-1265 expression following transfection (Fig. 1f and g). A CCK-8 assay was performed to explore the effect of miR-1265 expression on cellular proliferation. MKN45 cells treated with the miR-1265 inhibitor displayed a dramatically higher growth rate than the control. In contrast, SGC7901 cells treated with the miR-1265 mimic exhibited a lower growth rate than the control (Fig. 2a and b). Next, an EdU incorporation assay was performed, and the results indicated that miR-1265 overexpression in SGC7901 cells exerted an inhibitory effect on the quantity of EdU-positive cells compared with that in the control, whereas suppression of miR-1265 in MKN45 cells showed the opposite effect (Fig. 2c and d). In addition, a colony formation assay was conducted to further confirm the effect of miR-1265 on cellular proliferation. The overexpression of miR-1265 significantly impaired the colony formation ability of SGC7901 cells. Conversely, the suppression of miR-1265 was associated with the opposite trend in MKN45 cells (Fig. 2e and f). A cell cycle analysis using flow cytometry was performed to explore the cell cycle interruption. The results revealed that MKN45 cells treated with the miR-1265 inhibitor exhibited a marked decrease regarding the proportion of cells in the G0/G1 phase. In contrast, SGC7901 cells treated with the miR-1265 mimics displayed the opposite effect (Fig. 2g and h). Furthermore, flow cytometry was used to examine the effects of miR-1265 on apoptosis. A higher rate of apoptosis was detected in SGC7901 cells treated with the miR-1265 mimics than in the control, whereas MKN45 cells treated with the miR-1265 inhibitor showed the opposite trend (Fig. 3a and Supplementary Figs. 1a and 1b). These observations indicate that miR-1265 was able to suppress cellular proliferation and induce cell cycle arrest in the G0/G1 phase and promote apoptosis in GC cells.

3.3. miR-1265 represses autophagy in GC cells

Autophagy has a dual effect on tumors [15,16]. Thus, we investigated the association between miR-1265 and autophagy in GC cells. Immunohistochemical staining of consecutive sections of GC specimens revealed that tumors with high miR-1265 levels expressed higher levels of p62 than tumors with low miR-1265 levels (Fig. 3b). As shown in Fig. 3c-d, and Supplementary Fig. 1c and d, we found that GFP/mRFP-LC3 dot accumulation was induced following the knockdown of miR-1265 but decreased following the overexpression of miR-1265. Notably, the knockdown of miR-1265 increased the level of LC3-II protein expression in the extracts of GC cells, whereas the level of SQSTM1 protein expression decreased. In GC cells overexpressing miR-1265, the opposite effects were observed (Fig. 3e and f). Transmission electron microscopy (TEM) revealed that the suppression of miR-1265 could promote autophagy, whereas miR-1265 overexpression resulted in the suppression of autophagy (Fig. 3g and h). Altogether, our results indicate that miR-1265 may decrease autophagy in GC cells.

3.4. CAB39 is a direct target of miR-1265 in GC cells

MiRNAs are considered to be negative regulators of mRNA translation [4,5]. Using the TargetScan prediction program (www.targetscan.org), CAB39 was selected as a potential target of miR-1265. Furthermore, we validated the role of CAB39 as a targeted gene of miR-1265 with dual luciferase reporter assays. Thus, both wild-type (WT) and mutated (MUT) 3′-UTR sequences of CAB39 were structured based on potential binding sites using pGL3 firefly luciferase reporter plasmids (Fig. 4a). MiR-1265 overexpression dramatically decreased luciferase activity when the WT 3′-UTR of CAB39 was co-transfected into SGC7901 cells compared to that in the control. Nevertheless, no marked inhibitory effect of miR-1265 on luciferase activity was observed following co-transfection with the MUT 3′-UTR of CAB39 (Fig. 4b). Furthermore, we observed that miR-1265 overexpression decreased CAB39 protein expression and mRNA levels in GC cells (Fig. 4c and d).

We next investigated the association between CAB39 and miR-1265 expression in GC by analyzing their expression levels in matched pairs of specimens. The results indicated that CAB39 expression was dramatically higher in the tumor specimens than in adjacent normal specimens (Fig. 4e and f), and there was an inverse correlation between CAB39 and miR-1265 expression (Fig. 4g). Next, the association between CAB39 expression and the clinicopathological characteristics was analyzed in the same 63 patients with GC. The results indicated that CAB39 expression was positively correlated with tumor size (Table 1). These data suggest that CAB39 is a direct target of miR-1265 in GC cells.

To analyze the role of CAB39 in GC, we used the KM-plotter

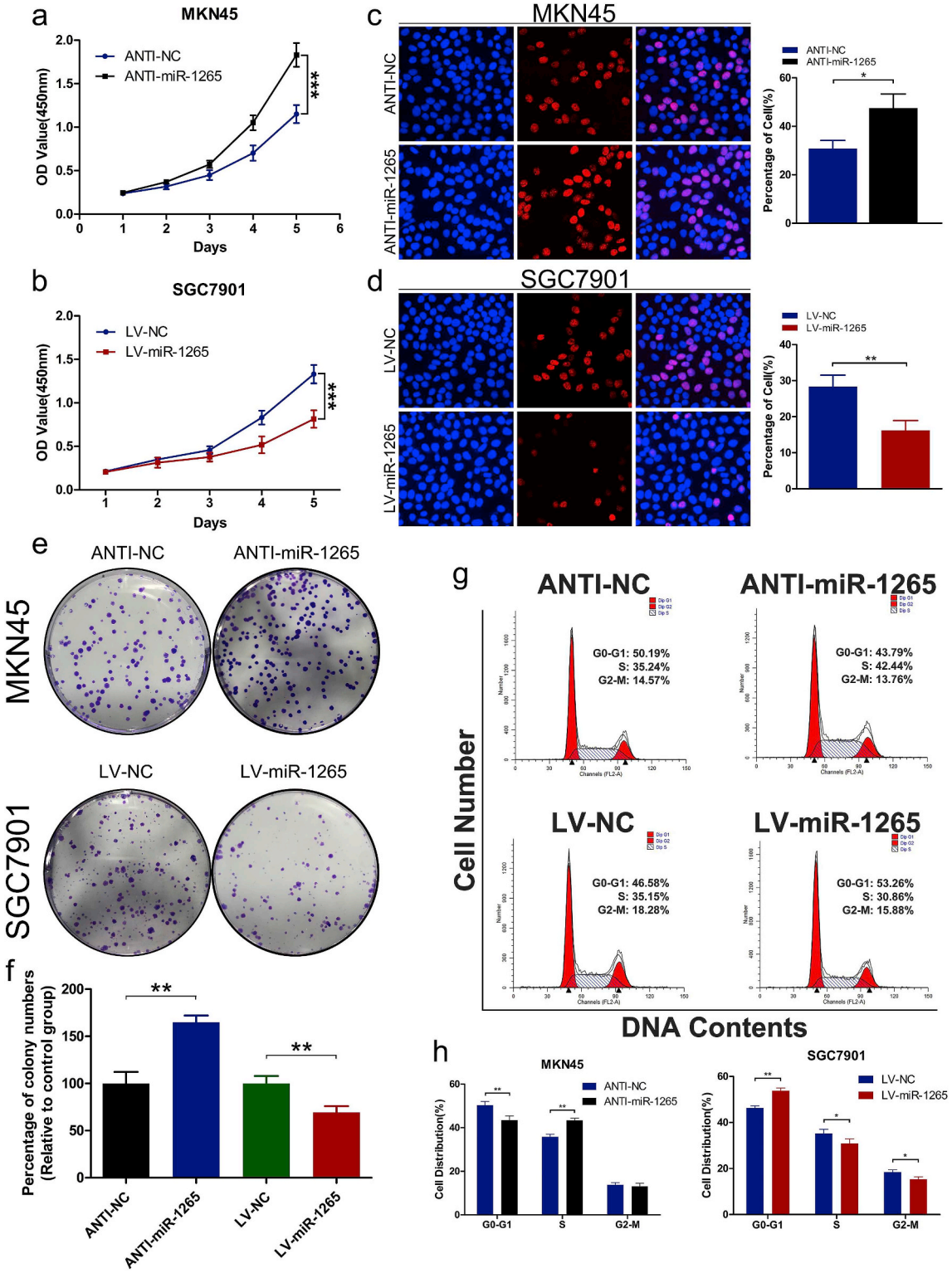

Fig. 2. MiR-1265 inhibits the proliferation of GC cells. (a,b) A CCK-8 assay was performed to evaluate the rate of GC cell proliferation following treatment with miR-1265 mimics or inhibitor lentivirus. The OD value among different groups was found to be significantly different by two-way ANOVA. *p < 0.05; **p < 0.01; ***p < 0.001. The data are expressed as the mean ± SD. (c,d) An Edu assay was utilized to measure DNA synthesis. Edu-positive cells are labeled with red fluorescence; the total cells are labeled with blue fluorescence from Hoechst staining. (e,f) A colony formation assay of cells treated with the miR-1265 mimics or inhibitor lentivirus. (g,h) Roles of miR-1265 expression alteration on cell cycle distribution.

database, which contains a larger cohort with complete prognosis information, to generate a survival curve of patients with GC with low or high CAB39 expression [29]. We verified that higher CAB39 expression was associated with lower survival rates in patients with GC (Fig. 4h).

3.5. miR-1265 suppresses proliferation and promotes apoptosis via downregulating CAB39 expression in GC cells

To clarify the underlying mechanisms of miR-1265 in GC

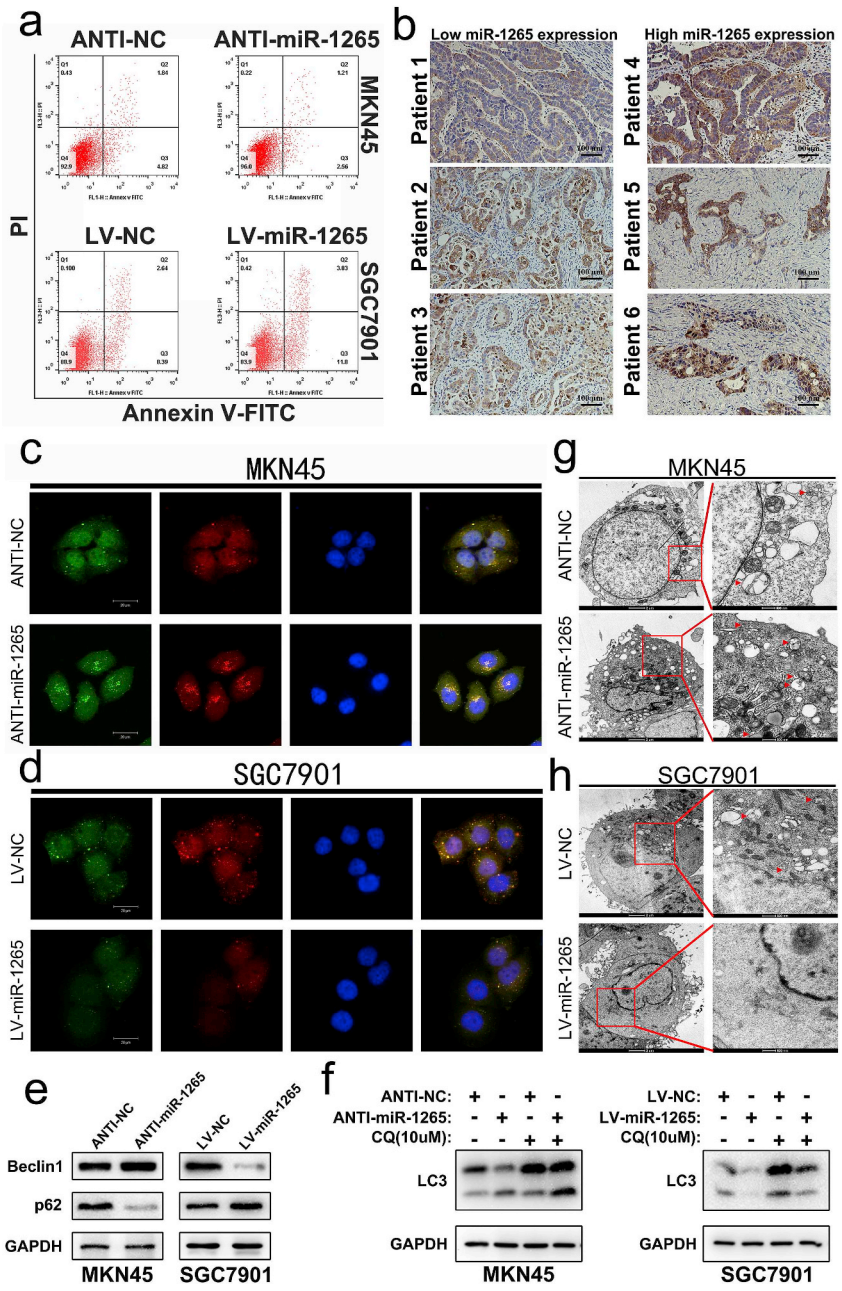

Fig. 3. MiR-1265 induces apoptosis and represses autophagy in GC cells. (a) The roles of altered miR-1265 expression on apoptosis. (b) Immunohistochemical staining of p62 in GC tissues. (c,d) Cells infected with a GFP-mRFP-LC3 lentivirus were seeded into confocal culture dishes, and yellow and red puncta were observed and counted using confocal microscopy (63× objective magnification; scale bar, 20 μm). (e,f) Western blot of the levels of BECN1, p62, and LC3-II protein expression after GC cells were transfected with miR-1265 mimics or inhibitor lentivirus. The cells were subsequently treated with chloroquine (CQ, 10 μM for 2 h) and collected for western blotting. (g,h) Transmission electron microscopy (TEM) was used to detect the autophagic microstructure of GC cells. Red arrows indicate a cellular autophagosome with a double-layer structure or an autolysosome generated via the fusion between an autophagosome and lysosome. (2500× and 8800× magnification; scale bar, 2 μm and 500 nm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

progression, we identified its gene target by validating the interaction between miR-1265 and CAB39 in GC cells. We demonstrated that the downregulation of CAB39 was able to rescue the effects of miR-1265 knockdown (Fig. 5a–f and Supplementary Figs. 2a–b). These findings indicate that miR-1265 suppresses proliferation and promotes apoptosis via directly downregulating CAB39 expression in GC cells.

3.6. CAB39 abrogates the adverse effects of miR-1265 on autophagy in GC cells

We further determined whether the suppressive effect of miR-1265 on autophagy was mediated by the suppression of CAB39 in GC cells. To investigate the association between CAB39 and autophagy, a Gene Set Enrichment Analysis was performed using CAB39 expression as a phenotype label. As shown in Fig. 6a and b, we found that a higher CAB39 expression was significantly correlated with autophagy pathway components ($P < 0.05$) in GC cohorts from the TCGA database. As shown in Fig. 6c, d, 6e, 6f, 6g, and 6 h, the western blotting and GFP/mRFP-LC3 dot accumulation analyses revealed that the overexpression of CAB39 partially restores autophagy inhibited by miR-1265. Similarly, the effects of miR-1265 knockdown were effectively counteracted by downregulating CAB39. Together, these data indicate that miR-1265 inhibits autophagy by downregulating CAB39 expression.

3.7. Autophagy mediated by the miR-1265/CAB39 axis is oncogenic and regulated by the AMPK-mTOR signaling pathway in GC cells

It has been reported that the AMPK/mTOR signaling pathway is associated with autophagy and that CAB39 plays a crucial role in this pathway [23–25]. Using western blotting, we confirmed that the phosphorylation of AMPK participated in miR-1265/CAB39-mediated autophagy. When miR-1265 was upregulated, the level of phosphorylated AMPK protein expression was significantly decreased, and the level of phosphorylated mTOR protein expression increased. Following CAB39 reconstitution, the effects of miR-1265 overexpression on the level of protein expression were reversed. Conversely, the

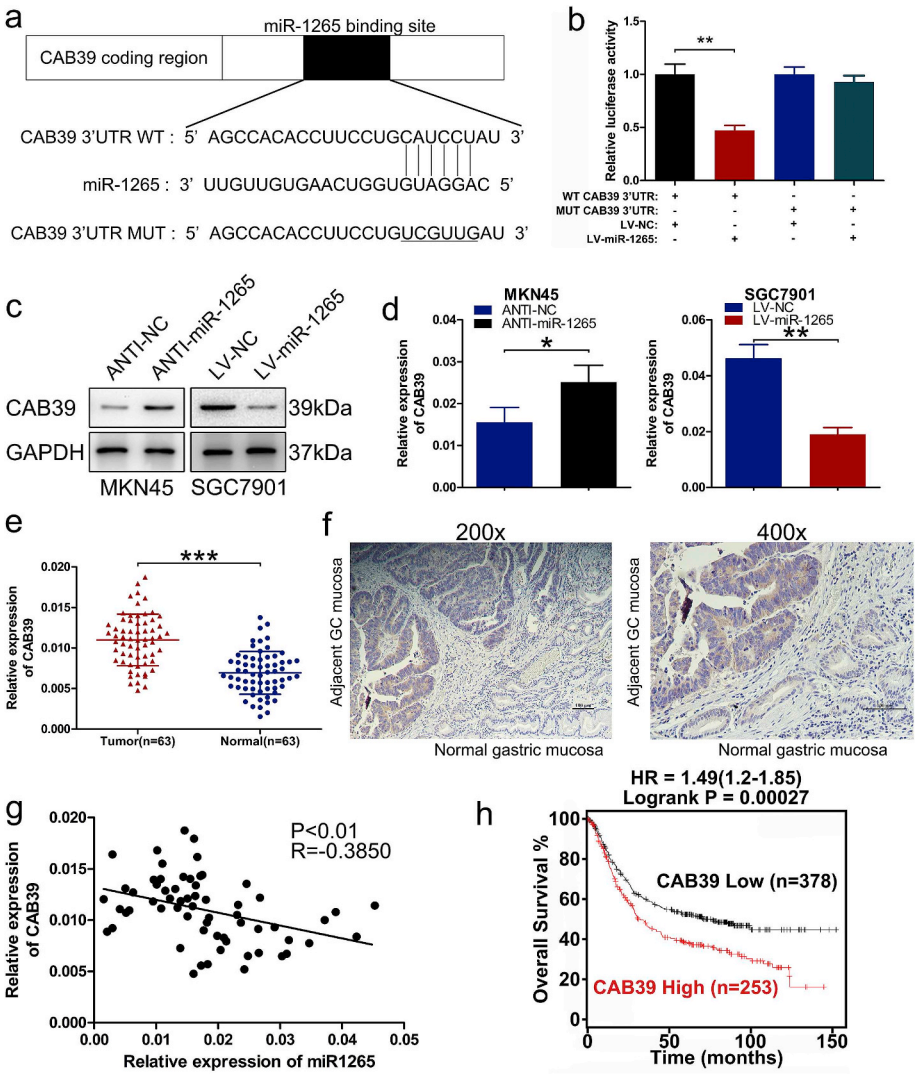

Fig. 4. CAB39 is a potential target gene of miR-1265 in GC. (a) The miR-1265 binding site in the 3′-UTR of CAB39 was identified by Targetscan and matched mutations. (b) A luciferase reporter assay was conducted to verify the interactions between miR-1265 and the CAB39 binding site in SGC7901 cells. (c) The level of CAB39 protein expression in GC cells transfected with miR-1265 mimics or the inhibitor lentivirus. (d) CAB39 mRNA expression in GC cells following transfection with miR-1265 mimics or the inhibitor lentivirus was assessed through qRT-PCR. (e) CAB39 expression in 63 pairs of GC specimens and matched adjacent gastric mucosa specimens was determined using qRT-PCR. (f) Immunohistochemistry staining was performed to evaluate the level of CAB39 protein expression in GC specimens and matched adjacent gastric mucosa specimens. (g) Negative correlation between miR-1265 and CAB39 expression in 63 pairs of GC specimens. (h) Overall survival analysis based on the levels of CAB39 expression in the KM-plotter database.

downregulation of miR-1265 resulted in the opposite effect (Fig. 6i and j). These results indicate that miR-1265 inhibits autophagy by reducing CAB39 expression, which inactivates AMPK and subsequently stimulates mTOR signaling.

To determine whether autophagy mediated by the miR-1265/CAB39 axis contributes to GC progression, we abrogated autophagy by inhibiting the expression of ATG12, an important factor associated with autophagosome elongation [30]. As shown in Fig. 7a, b, 7c, 7d, 7e, 7f, 7g, and 7h, ATG12 knockdown reversed the changes in cellular proliferation and apoptosis induced by miR-1265 knockdown or CAB39 overexpression. Western blotting was performed to verify that autophagy was inhibited after ATG12 knockdown (Fig. 7i). These data show that autophagy mediated by the miR-1265/CAB39 axis drives GC progression.

3.8. miR-1265 induces the growth inhibition of GC cells in vivo

To explore the role of miR-1265 in tumor growth in vivo, we subcutaneously injected GC cells into nude mice. The miR-1265-inhibited tumors were larger than the negative controls (MKN45 cells) (Fig. 8a and c). In contrast, the tumors overexpressing miR-1265 were significantly smaller than the negative controls (SGC7901 cells) (Fig. 8b and d). The Ki-67 distribution revealed a lower proliferation index in the miR-1265-overexpressing group compared to that in the negative controls, whereas the miR-1265 knockdown group displayed the opposite effects (Fig. 8e). Moreover, A TUNEL assay indicated that the miR-1265-overexpressing group had a higher apoptotic index than the negative controls. In contrast, the miR-1265 knockdown group exhibited the opposite trend (Fig. 8f). In addition, western blotting and immunohistochemical analyses revealed a marked downregulation of CAB39 expression in the miR-1265-overexpressing group compared with that in the negative controls. The opposite trends were observed in the miR-1265 knockdown group (Fig. 8g). Taken together, these findings indicate that miR-1265 can effectively reduce tumor growth by interfering with oncogenic autophagy.

3.9. miR-1265 inhibits the growth of human gastric organoids

Organoids dependably recapitulate many aspects of the tissue from which they are derived. Therefore, organoids allow us to directly investigate some pathologies of human tissues [31,32]. Human gastric organoids were constructed from fresh stomach biopsies of patients, and HE staining was performed (Fig. 8h). We evaluated miR-1265 expression following transfection with miR-1265 mimics or inhibitor lentivirus using qRT-PCR (Fig. 8i). The diameters of 30 organoids in each group were measured. We found that miR-1265 overexpression inhibits the growth of gastric organoids, whereas miR-1265 knockdown results in the opposite trend (Fig. 8j and k).

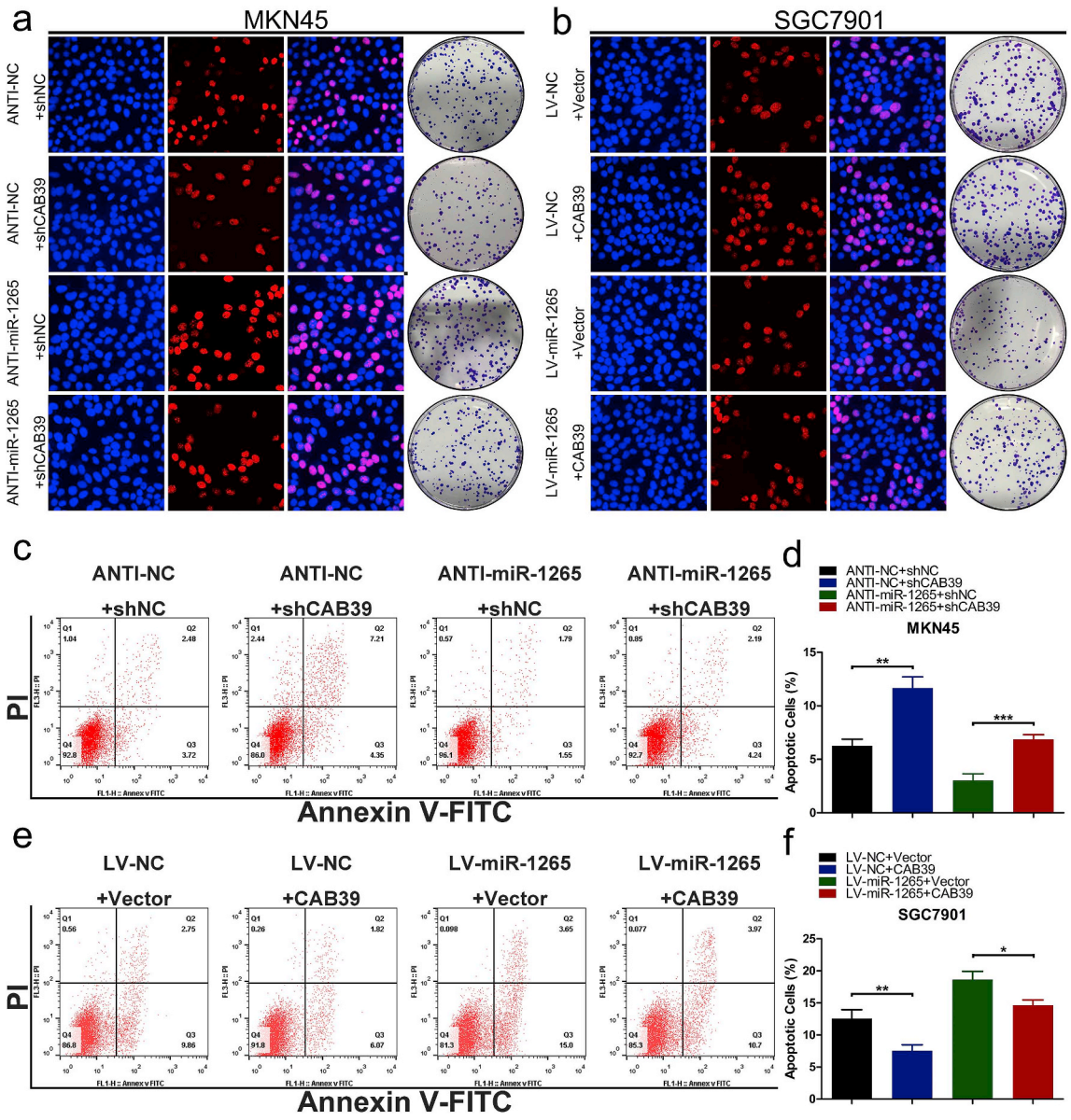

Fig. 5. MiR-1265 decreases cellular proliferation and promotes apoptosis by inhibiting CAB39 expression. (a,b) An EdU incorporation and colony formation assay were conducted to evaluate cellular proliferation. Rescue experiments for miR-1265 inhibition were conducted by downregulating CAB39 in MKN45 cells. Rescue experiments for miR-1265 overexpression were conducted via the ectopic expression of CAB39 without the 3′-UTR in SGC7901 cells. (c,d,e,f) Apoptosis was detected using flow cytometry (c,d: MKN45; e,f: SGC7901).

4. Discussion

MiRNAs can function as post-transcriptional regulators of gene expression in GC and might be useful as novel therapeutic strategies against GC [33]. In the present study, we demonstrated, for the first time, that miR-1265 expression is lower in GC specimens than in the adjacent normal specimens. Moreover, miR-1265 was found to play a tumor-suppressive role in GC, suppressing cellular proliferation, inducing cell cycle arrest, and promoting apoptosis.

There is increasing evidence that cancers can use autophagy to meet their increased metabolic demand for growth and proliferation [34]. For example, Alicia et al. found that autophagy activation promotes cellular proliferation in prostate cancer [35]. Similarly, Gu et al. verified that oncogenic autophagy occurs in pancreatic cancer [28]. In the underlying mechanisms of tumor promotion, it is thought that metabolic stress necessitates the activation of autophagy to maintain mitochondrial function and energy homeostasis [36–38]. Here, we found that miR-1265 expression led to the inhibition of autophagy in GC cells.

To investigate the underlying mechanisms associated with miR-1265, putative targets of miR-1265 were predicted using a bioinformatics analysis. Using a fluorescent reporter assay, CAB39 was confirmed to be a direct target of miR-1265. It has been reported that CAB39 is a highly evolutionarily conserved protein that has an extended α-helical repeat rod-like structure [21,39,40]. In addition, CAB39 plays a crucial role in the LKB1-STRAD-CAB39 complex [21,41], in which CAB39 functions to stabilize and relocalize the complex from the nucleus to the cytoplasm [42]. Data from both Woods et al. and Hawley et al. demonstrated that the binding of LKB1 to STRAD and CAB39 increased AMPK phosphorylation at Thr172 by over 100-fold [43,44].

It has been reported that CAB39 is an oncogenic factor in hepatocellular carcinoma, pancreatic cancer, and glioma [26–28]. Herein, we found that CAB39 expression was higher in GC specimens than in adjacent normal specimens in our patient cohort. MiR-1265

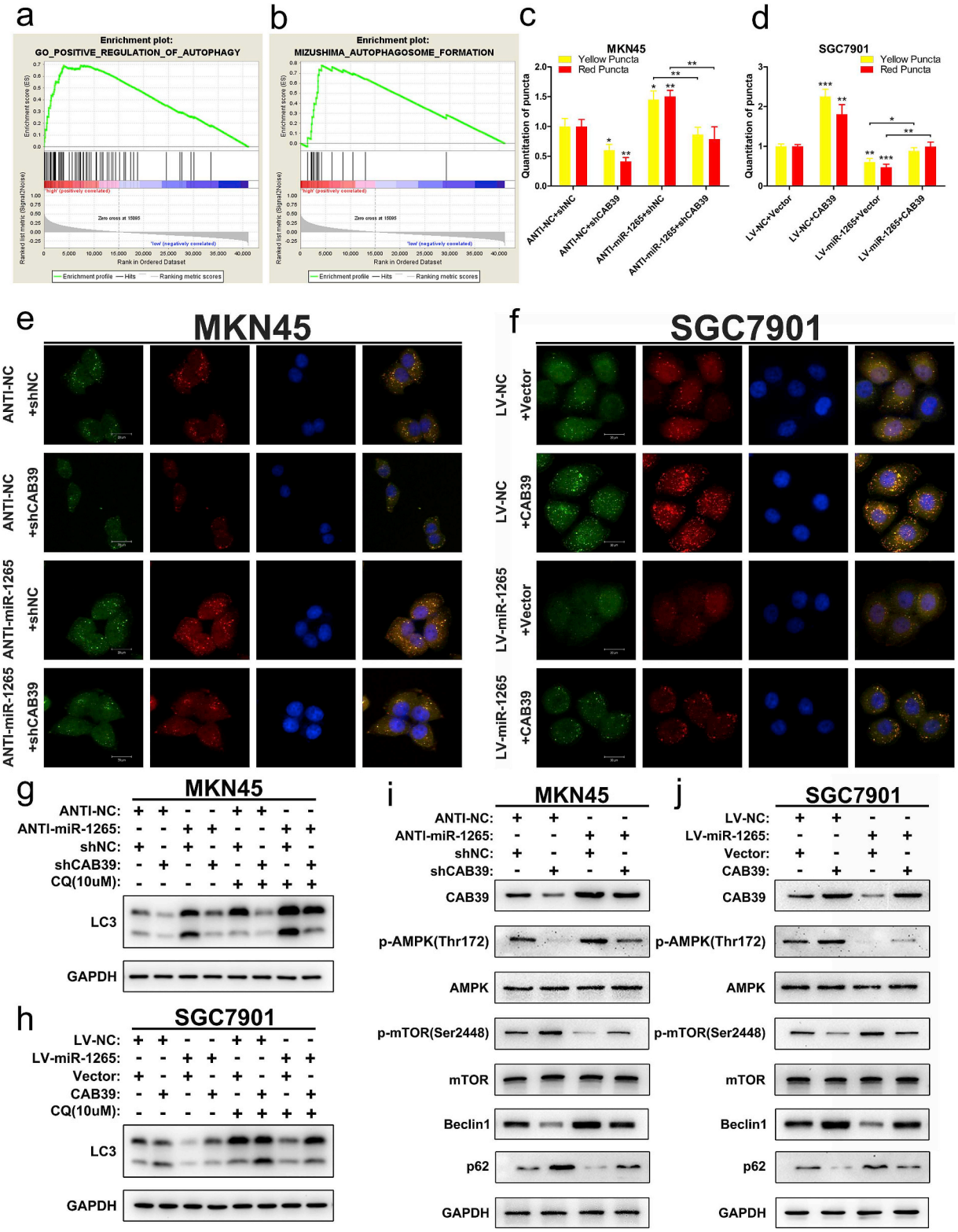

Fig. 6. MiR-1265 suppresses autophagy by inhibiting CAB39 expression. (a,b) A gene set enrichment analysis (GESA) was used to compare the CAB39 higher group (red) against the CAB39 lower group (blue) among the GC cohorts in the TCGA data set. Higher CAB39 expression correlates with the positive regulation of autophagy and autophagosome formation. (c,d,e,f) Following transfection with GFP-mRFP-LC3, the cellular puncta were photographed using confocal microscopy and enumerated as described in Fig. 4. Rescue experiments for miR-1265 inhibition were conducted by downregulating CAB39 in MKN45 cells. Rescue experiments for miR-1265 overexpression were conducted by ectopically expressing CAB39 without 3′-UTR in SGC7901 cells. (g,h) Western blotting yielded results consistent with the confocal imaging of MKN45 and SGC7901 cells (CQ, 10 μM for 2 h). (i,j) A Western blot analysis was performed to evaluate the levels of protein expression in the CAB39/AMPK/mTOR pathway in GC cells (f: MKN45; g: SGC7901). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

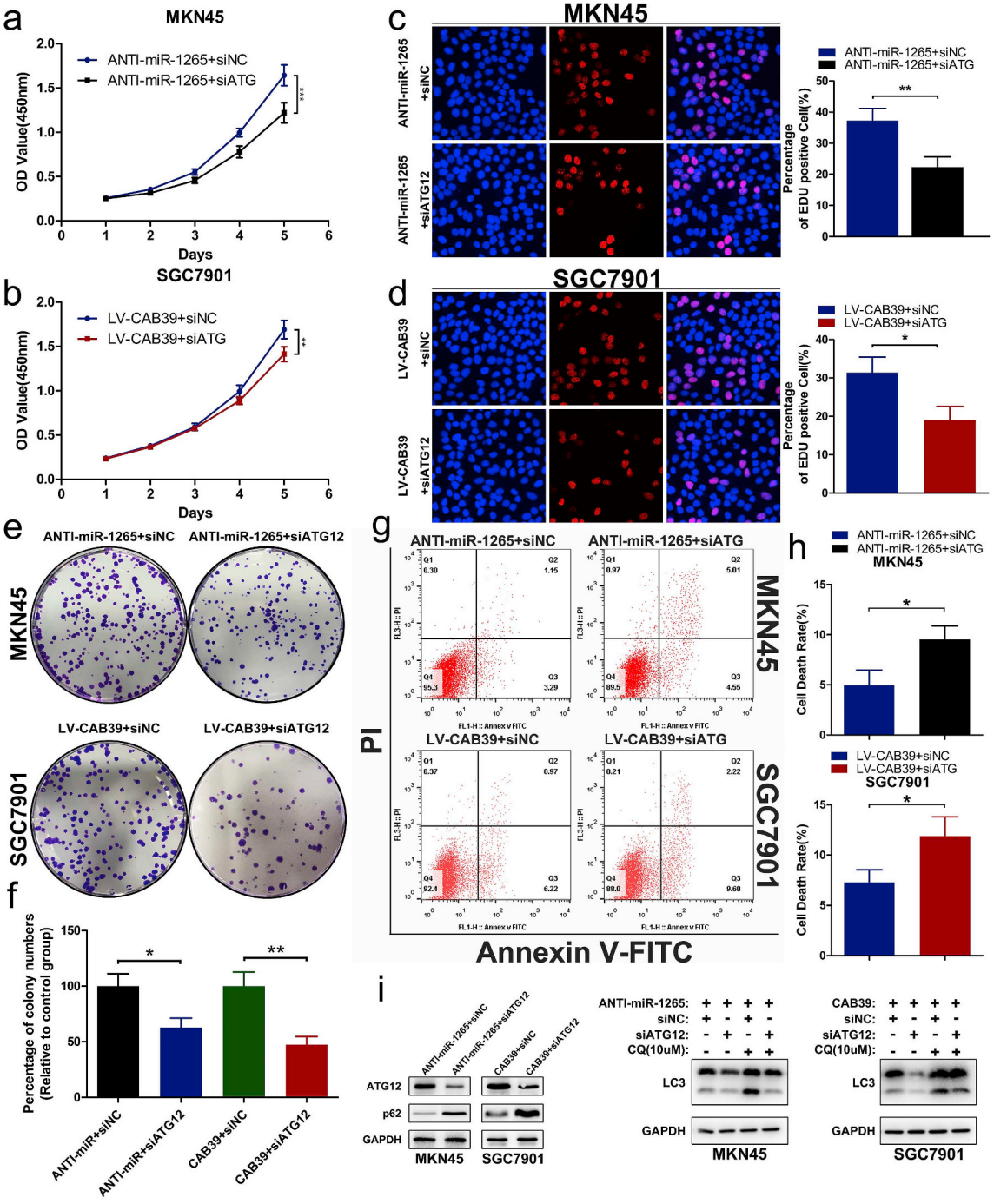

Fig. 7. Autophagy mediated by the miR-1265/CAB39 axis is oncogenic in GC cells. (a–h) Knockdown of Atg12 significantly decreased cell viability according to CCK8 assay (a,b), an Edu assay (c,d), colony formation ability (e,f), and induced apoptosis (g,h) of GC cells following the downregulation of miR-1256 or overexpression of CAB39. (i) Western blot indicating that the knockdown of Atg12 significantly suppressed autophagy.

overexpression suppressed CAB39 expression through degrading CAB39 mRNA, and ectopic CAB39 expression markedly reversed the effects induced by miR-1265. In addition, the downregulation of CAB39 expression impaired the increased proliferation and autophagy and decreased apoptosis induced by the knockdown of miR-1265. Furthermore, miR-1265 negatively regulated the AMPK signaling pathway through downregulating CAB39 expression and further led to the inhibition of autophagy. Taken together, our results suggest that the biological functions of miR-1265 in GC are mediated through the downregulation of CAB39.

ATG12 is an ubiquitin-like modifier involved in the elongation of pre-autophagosomal structures [45,46]. In the present study, the knockdown of ATG12 impaired the cellular proliferation and resistance to apoptosis induced by miR-1265 knockdown and ectopic expression of CAB39. Our results indicated that miR-1265 functions to decrease proliferation and induce apoptosis by suppressing oncogenic autophagy in GC cells. In addition, we found that the autophagy induced by the CAB39-LKB1-AMPK axis is oncogenic in GC cells.

With the development of 3D culture technology, it is possible to witness the remarkable self-organizing properties of embryonic and adult mammalian stem cells; moreover, the resulting organoids reflect the dominant properties of their respective organs. Therefore, organoid technology can be used to model the development of various human pathologies [31,47]. In the present study, we constructed gastric

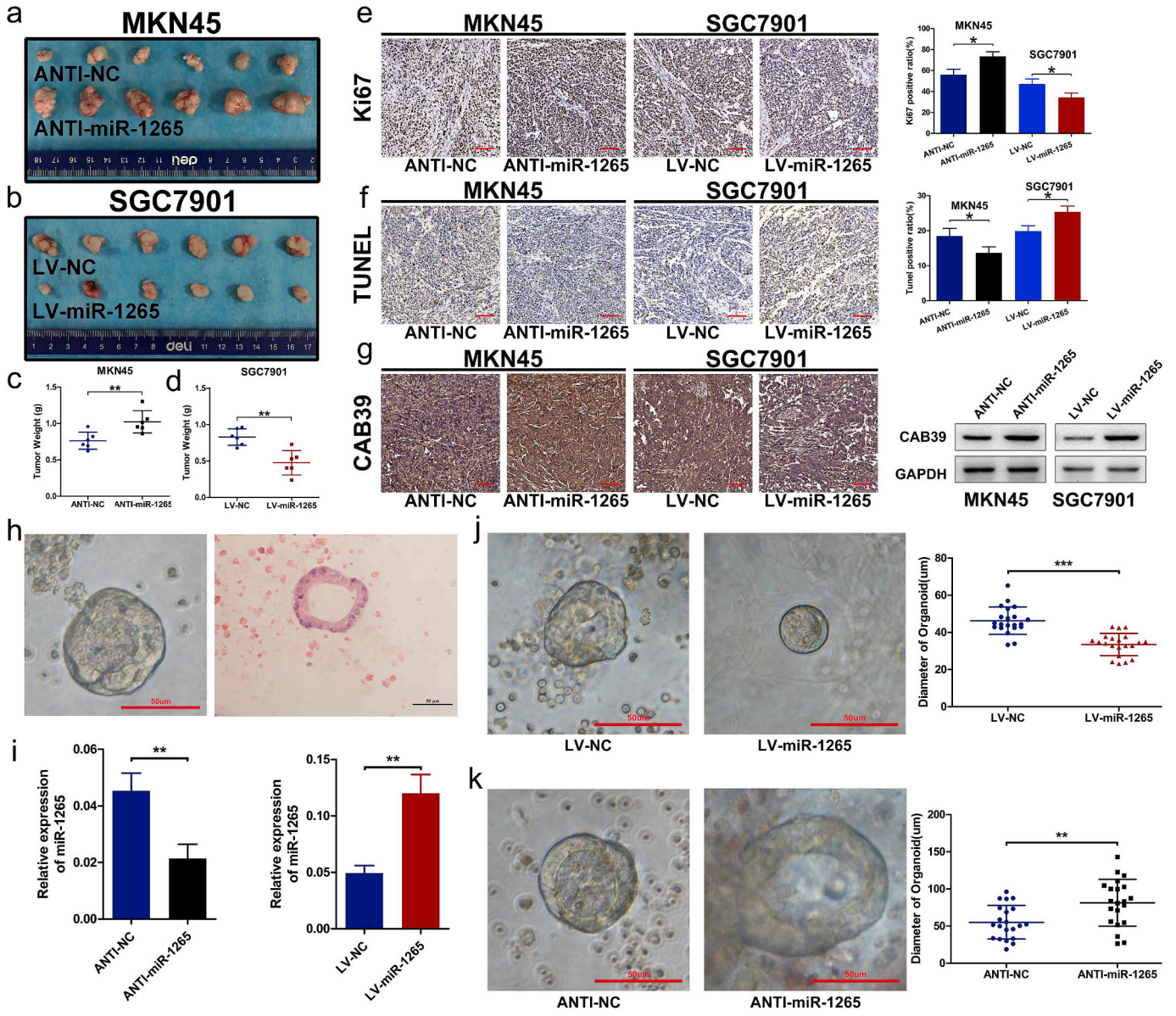

Fig. 8. MiR-1265 suppressed the growth of both GC cells in vivo and human gastric organoids. (a,b,c,d) Tumor size and weight of nude mice injected with the indicated cells; n = 6 mice/group. (e) Ki-67 staining indicated that miR-1265 inhibited cellular proliferation in vivo. Scale bar: 50 μm. (f) A TUNEL assay was conducted to evaluate apoptosis in vivo. Scale bar: 50 μm. (g) Western blotting and immunohistochemical analyses were performed to evaluate CAB39 expression in vivo. Scale bar: 50 μm. (h) Human gastric organoids were successfully established, and HE stained samples were imaged. Scale bar: 50 μm. (i) MiR-1265 expression in human gastric organoids following transfection with miR-1265 mimics or inhibitor lentivirus. (j,k) The effect of altered miR-1265 expression on the size of human gastric organoids. Scale bar: 50 μm.

organoids from freshly isolated human stomach tissues. Using Ki-67 staining and measuring the organoid diameter, we found that miR-1265 suppressed organoid growth; however, further research is required to investigate the associated mechanisms.

Taken together, our results indicate that miR-1265 plays a critical role in GC progression. The autophagy mediated by the miR-1265/CAB39 axis exerts oncogenic effects. Moreover, these findings suggest that the miR-1265/CAB39 axis might be a useful therapeutic target for GC in the future because autophagy provides cells with the nutrients required by fast-growing tumors.

Conflicts of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

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