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VMP1 related autophagy and apoptosis in colorectal cancer cells: VMP1 regulates cell death

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ABSTRACT

Vacuole membrane protein 1 (VMP1) is an autophagy-related protein and identified as a key regulator of autophagy in recent years. In pancreatic cell lines, VMP1-dependent autophagy has been linked to positive regulation of apoptosis. However, there are no published reports on the role of VMP1 in autophagy and apoptosis in colorectal cancers. Therefore, to address this gap of knowledge, we decided to interrogate regulation of autophagy and apoptosis by VMP1. We have studied the induction of autophagy by starvation and rapamycin treatment in colorectal cell lines using electron microscopy, immunofluorescence, and immunoblotting. We found that starvation-induced autophagy correlated with an increase in VMP1 expression, that VMP1 interacted with BECLIN1, and that siRNA mediated down-regulation of VMP1-reduced autophagy. Next, we examined the relationship between VMP1-dependent autophagy and apoptosis and found that VMP1 down-regulation sensitizes cells to apoptosis and that agents that induce apoptosis down-regulate VMP1. In conclusion, similar to its reported role in other cell types, VMP1 is an important regulator of autophagy in colorectal cell lines. However, in contrast to its role in pancreatic cell lines, in colorectal cancer cells, VMP1-dependent autophagy appears to be pro-survival rather than pro-cell death.

1. Introduction

Colorectal cancer (CRC) is one of the most common digestive cancers worldwide. Although the prognosis of CRC has improved in recent years because of combined therapy, the prognosis of advanced CRC with lymphatic metastasis remains poor because there is no efficacious therapy for advanced CRC [1]. Resistance to chemotherapy is a vital problem associated with poor prognosis and problems with treatment [2]. Substantial evidence indicates that autophagy is a mechanism contributing to chemoresistance in colorectal cancer cells [3].

Autophagy is an evolutionarily conserved process that plays a role in the turnover of old organelles and proteins to obtain energy. The role of autophagy in tumors is contradictory. There is evidence describing the different roles of autophagy depending on the type of tumor [4–8]. In colorectal cancer cells, more evidence tends to describe autophagy as a pro-survival tumor cell behavior, enhancing the aggressiveness of tumor cells and their ability to adapt to apoptotic stimuli, starvation or anti-tumor drugs [9,10].

Autophagy is regulated by specific genes known as ATGs (autophagy-related genes). Many ATG proteins have been identified, including vacuole membrane protein 1 (VMP1), which is very active in acinar cells during acute pancreatitis [11]. VMP1 interacts with BECLIN1 through its target lipid kinase Vps34/PI3KC3 to assemble a class III PI3K complex, which positively regulates the formation of autophagosomes.

To date, several reports have demonstrated anti-tumor functions of VMP1. In hepatocellular carcinoma, VMP1 has been shown to inhibit proliferation and metastasis [13], and in pancreatic cell lines, inhibition of VMP1 decreased apoptosis [14]. Though there is evidence that in non-small lung cancers, expression of VMP1 has been linked to poor prognosis [12]. To our knowledge, a pro-tumorigenic role of VMP1 has not been reported in colorectal cancer.

Given the lack of any report on the role of VMP1 in colorectal cancers, we decided to test how VMP1 regulates autophagy and

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apoptosis in this type of cancer. We used several different colorectal cancer cell lines to confirm whether autophagy occurs in these cells when under stress or autophagic-inducing conditions and to determine whether VMP1 regulates autophagy in these cells by interacting with BECLIN1. This is the first report investigating the role of VMP1 in colorectal cancer and the first one to demonstrate a pro-survival (and presumably pro-tumorigenic) role of the gene.

2. Methods and materials

2.1. Cells and culture conditions

SW480 cells were cultured in Leibovitz's L-15 medium (Invitrogen, Carlsbad, CA, USA) with 10% FBS at 37 °C in a humidified atmosphere of 5% CO_2. The cells were treated with etoposide (Sigma–Aldrich, Shanghai, China) at 5 μg/ml or the apoptosis-inducing agent staurosporine (Sigma–Aldrich, Shanghai, China) at 2 μM, unless otherwise indicated. Rapamycin (Invitrogen, Carlsbad, CA, USA) was used as indicated. All of the agents were dissolved in DMSO.

2.2. RNA interference

VMP1-siRNA#1 (described previously [12]) and VMP1-siRNA#2 (sense: 5′ GGCAUCGUCAAAGCAUUGUTT 3′, antisense: 5′ ACAAUGCUUUGACGAUGCCTT 3′) were purchased from Genepharma (Genepharma, Shanghai, China). The cells were transfected with 100 pmol VMP1 siRNA in six-well plates using Lipofectamine RNAi max (Invitrogen, Carlsbad, CA, USA). Negative control siRNA was transfected under the same conditions. Then, the cells were plated at 20×10^4 cells per well in six-well plates and cultured with the indicated agents. The changes in protein levels were measured by Western blotting at 48 h after transfection.

2.3. Western blot analysis

Western blotting was performed as previously described [13]. Anti-VMP1, anti-BECLIN1, anti-LC3, anti-cleaved PARP, anti-cleaved Caspase-3, anti-pro Caspase-3, and anti-β-actin antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). The band intensity was semi-quantified by BandScan software (Bio-Rad, Shanghai, China) after digitizing with a V300 scanner (Epson, Tokyo, Japan).

2.4. Immunofluorescence staining

The cells were fixed in 4% paraformaldehyde for 10 min and then washed with PBS for 5 min. The slides were immersed in 100 μg/ml digitonin for 15 min at room temperature and then washed. Next, the cells were incubated with an anti-LC3 antibody (MBL, Nagoya, Japan) and washed extensively. FITC-conjugated anti-rabbit IgG (MBL, Nagoya, Japan) was added to the cells, and the cells were washed again. Autophagosomes were examined using a fluorescence microscope (Nikon Eclipse TE200 microscope, Nikon, Inc., Melville, NY, USA).

2.5. Cell viability assays

Cell viability assays were performed as previously described [14]. Each assay was performed in triplicate, and the average absorbance was calculated.

2.6. Apoptosis analysis

An annexin-V-PE Apoptosis Detection kit (Invitrogen, Carlsbad, CA, USA) was used to measure apoptosis. The cells were washed with PBS and resuspended in 1 × Binding Buffer at a concentration of 1×10^6 cells/ml. Subsequently, 5 μl of annexin-V-PE and 5 μl of PI were added to 100 μl of the cell suspension and incubated for 15 min in the dark. After incubation, 400 μl of 1 × Binding Buffer was added. The analyses were performed using a FACScan flow cytometer (Beckman Instruments, Fullerton, CA, USA).

2.7. Electron microscopy

SW620 cells were cultured in serum-free medium for 2, 4 or 8 h. The cells were fixed with 2% paraformaldehyde-2% glutaraldehyde in 0.1 ml/L phosphate buffer (pH 7.4), followed by 1% osmium tetroxide. After dehydration, thin sections were stained with uranyl acetate and lead citrate for observation using a JEM 100 CX electron microscope (JEOL, Peabody, NY, USA).

2.8. Co-immunoprecipitation assays

The supernatants from lysates of colorectal cancer cells were incubated with anti-sera bound to protein A-Sepharose beads (Amersham Bioscience) for 2 h at 4 °C and washed extensively. Bound proteins were eluted with 100 mM glycine–HCl, pH 2.5, precipitated by 5% trichloroacetic acid, washed with ice-cold acetone and resuspended in SDS sample buffer.

2.9. Statistical analysis

Statistical analysis was performed by SPSS (SPSS Inc., Chicago, USA). Statistical significance was determined using Student's *t*-test. A *p*-value equal to or less than 0.05 was considered significant.

3. Results

3.1. Serum deprivation-induced autophagy is associated with an upregulation of VMP1 in colorectal cancer cell

To confirm that serum starvation or rapamycin treatment would induce autophagy in colorectal cancer cells, SW480 cells were treated will serum-free medium or rapamycin and were examined by transmission electron microscopy (TEM). Starvation and rapamycin treatment strongly induced autophagy as judged by formation of autophagosomes in Fig. 1A. The microtubule-associated protein 1 light chain 3 (LC3) is a homologue of Apg8p for autophagy in yeast, which is a marker of autophagy formation. The C-Terminal fragment of LC3 is cleaved immediately to yield a cytosolic form called LC3-I. A subpopulation of LC3-I is further converted to an autophagosome-associating form, LC3-II, which can be detected by Western blot [15]. Immunoblots were used to measure the levels of LC3 protein. As shown in Fig. 1B and C, the incubation of SW480 cells with serum-free media or rapamycin was associated with an increase in the ratio of LC3-II /LC3-I. We observed that the VMP1 levels increased during induction of autophagy and were positively correlated with time (starvation) and with rapamycin-dependent induction of VMP1 protein expression in a dose-dependent manner (Fig. 1D and E).

These data show that autophagy is triggered by starvation and rapamycin and that VMP1 is associated with autophagy.

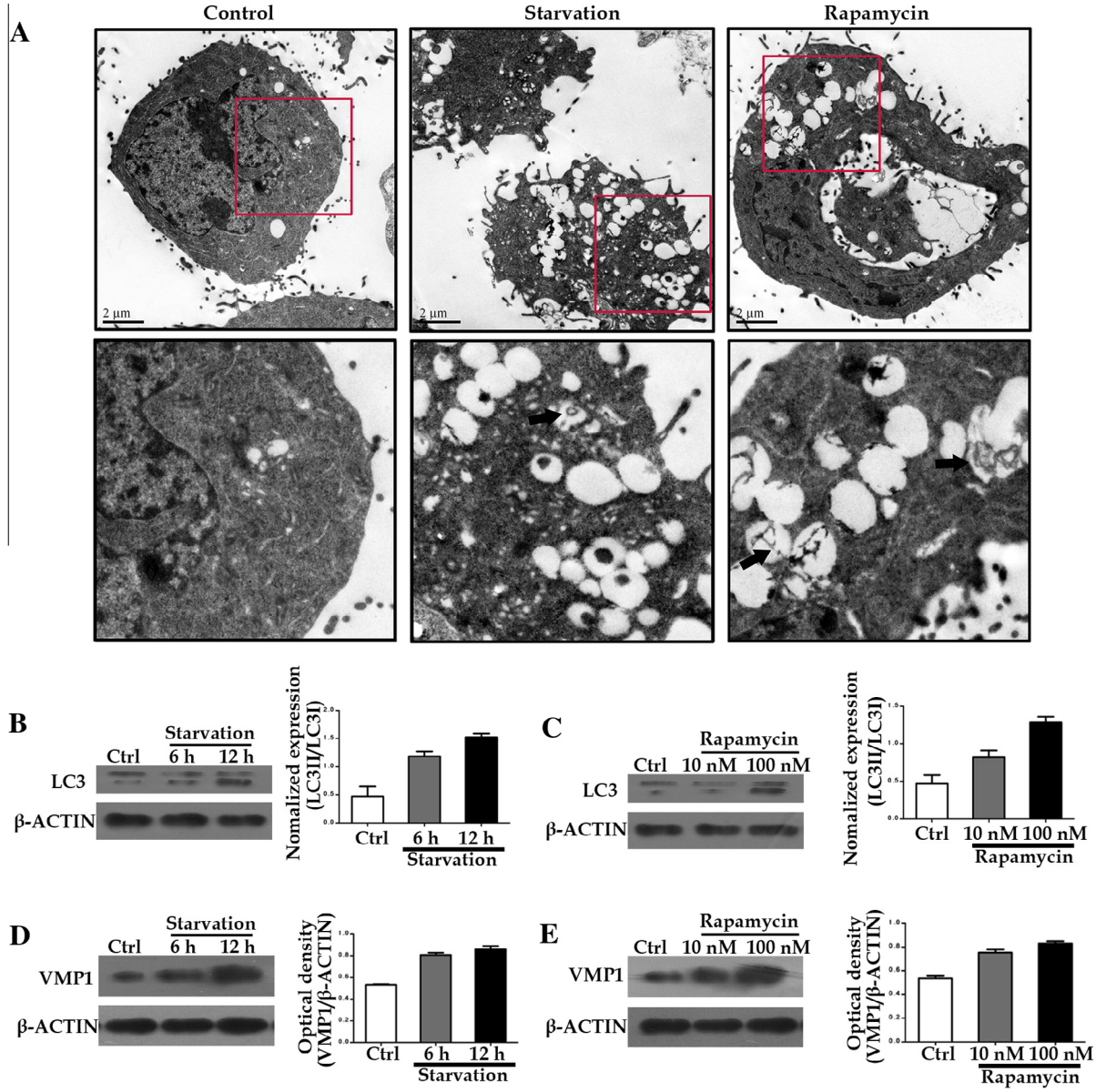

Fig. 1. Autophagy is induced by starvation in SW480 cells. (A) Electron micrographs of starvation-induced (12 h) or rapamycin-induced (100 nM) autophagy in SW480 cells. (B) Western blot of LC3 after serum-free induction at indicated time points. Right: quantitation of the expression data. (C) Western blot of LC3 after rapamycin treatment at the indicated concentrations. Right: quantitation of the expression data. (D and E) VMP1 Western blot and its quantification in SW480 cells after serum-free or rapamycin treatment as indicated. The data represent the means ± SD from three independent experiments.

3.2. VMP1 expression is decreased during apoptosis induced by staurosporine

Various pro-autophagic proteins also play a role in promoting apoptosis [16,17]. The net effect of these opposing cellular processes may ultimately serve to inhibit or promote autophagy, thereby leading to cell death or survival. Therefore, we decided to investigate the relationships between VMP1 and apoptosis in colorectal cancer cell lines. First, we established an apoptotic model for SW480 using staurosporine. As shown in Fig. 2A, cleaved Caspase-3 and cleaved PARP, inducers of apoptosis, were activated after treatment with staurosporine for different lengths of time. Simultaneously, a decrease in the protein levels of VMP1 in the presence of staurosporine was observed (Fig. 2B). However, we did not observe changes in the levels of BECLIN1, which has been reported by others to decrease during apoptosis [18,19]. Next, we used a different dose to treat SW480 cells (Fig. 2C and D). Both time-dependent and dose-dependent VMP1 downregulation were observed in SW480 cells. Thus, the evidence suggests that VMP1 may play a role during the apoptotic process in colorectal cancer cells.

3.3. Serum deprivation leads to cell death when VMP1 is inhibited

VMP1 is an ATG protein with recently reported autophagy-related apoptosis in pancreatic cancer cells [20]. However, based on our evidence of the down-regulation of VMP1 during apoptosis, we hypothesized that VMP1 is a negative regulatory factor for apoptosis in colorectal cell lines. To verify this hypothesis, we knocked down VMP1 in SW480 cells using siRNA (Fig. 3A and B). SW480 cells were more sensitive to inducers of apoptosis (Fig. 3C and D) and that the cell viability was markedly reduced

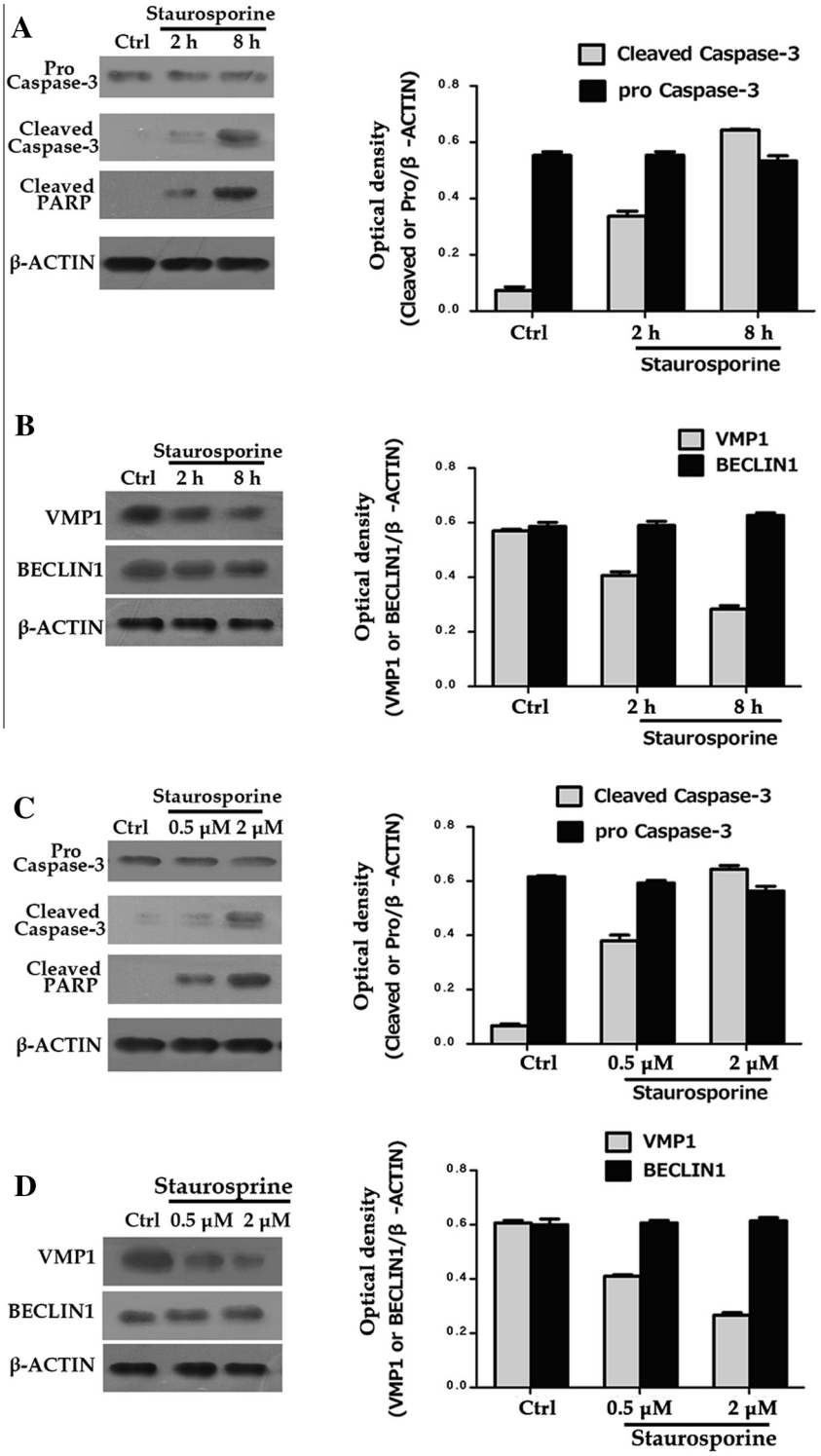

Fig. 2. VMP1 is downregulated when apoptosis is activated. (A and B) Western blot and corresponding optical density quantification of pro-caspase-3, cleaved caspase-3, cleaved PARP, VMP1, and BECLIN1 after treatment with staurosporine for 2 or 8 h. (C and D) Western blot and corresponding optical density quantification of pro-caspase-3, cleaved caspase-3, cleaved PARP, VMP1 and BECLIN1 after treatment with 0.5 or 2 μM staurosporine. The data represent the means ± SD from three independent experiments.

in the nutrient-starved VMP1 siRNA group (Fig. 3E) compared to the nutrient-starved negative siRNA-treated control group. These results indicate that VMP1 may be an anti-apoptotic factor in colorectal cell lines. On the other hand, to further confirm the promotion function of VMP1 in autophagy, untreated SW480 cells and siVMP1 cells were cultured in serum-free medium. Immunofluorescent staining for LC3 revealed that VMP1 knockdown significantly reduced autophagosome formation (Fig. 3F and G). These data suggest that VMP1 has a pro-autophagic function in colorectal cell lines.

3.4. VMP1 triggers autophagy through binding to BECLIN1

The BECLIN1-Class III PI3K complex is thought to initiate autophagocyte formation, and an association between BECLIN1 and VMP1 has been demonstrated in non-colorectal cancer cell lines

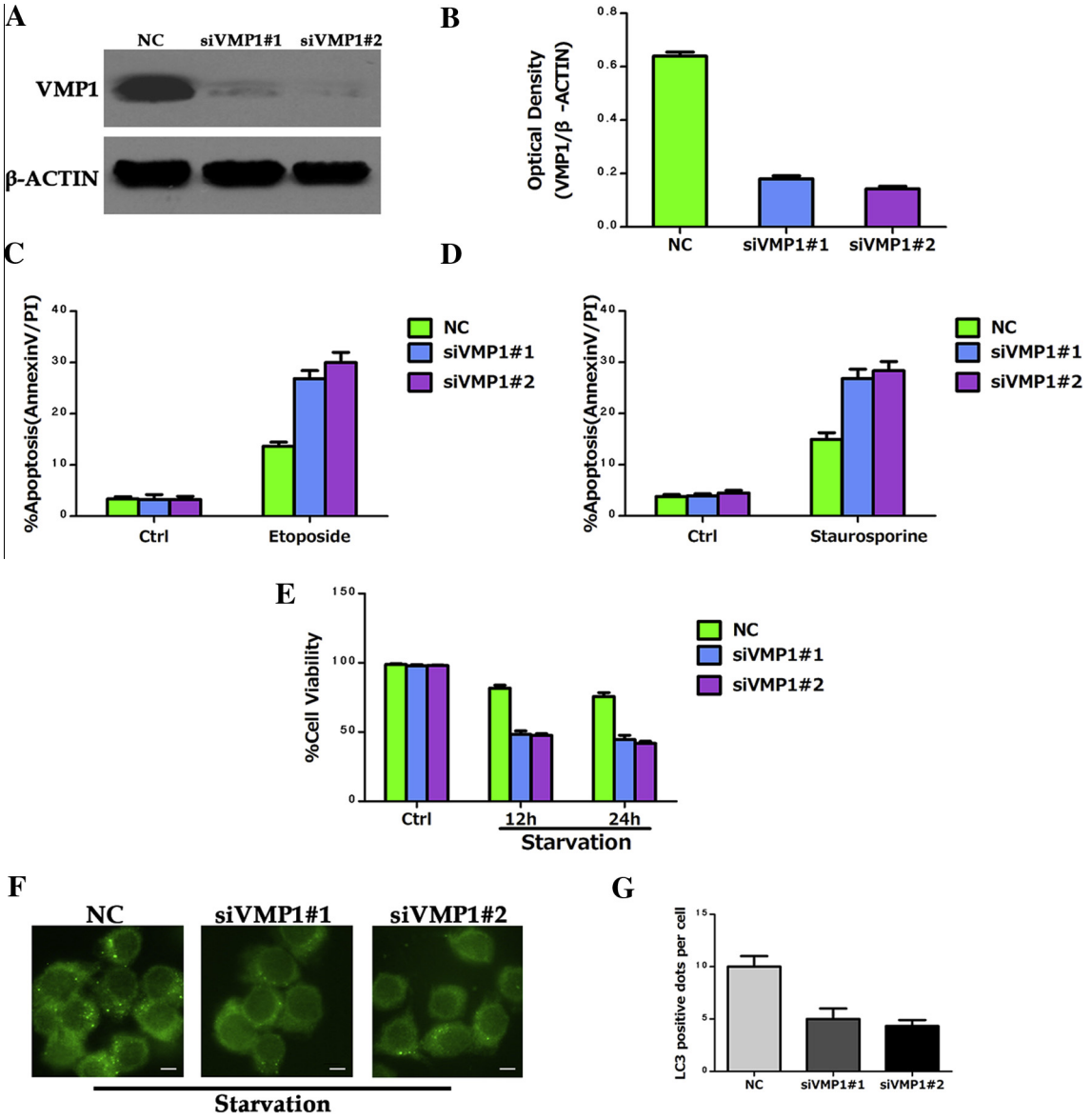

Fig. 3. VMP1 downregulation suppresses autophagy and sensitizes to apoptosis. (A and B) Western blot analysis and optical density quantification of VMP1 levels in SW480 cells after transient transfection with negative control and VMP1-siRNA oligonucleotides. (C and D) Flow cytometry analysis of apoptosis rates of transduced SW480 cells VMP1 or noncoding siRNA SW480 cells treated with indicated concentrations of staurosporine (2 μM, 6 h) or etoposide (5 μg/ml, 24 h). (E) Cell viability of SW480 cells cultured in serum-free medium for 12 or 24 h as determined using an MTT assay. The data represent the means ± SD of three independent experiments ($p < 0.05$). (F) Staining for LC-3 of SW480 cells treated under nutrient-deprivation. (G) Quantitation of data in 3F (10,000 cells were counted per experiment). Size bars represent 5 nm. The data represent the means ± SD of three independent experiments.

[12]. However, whether this association can be also observed in colorectal cancer cells has not been investigated. Therefore, we observed the mechanisms by which VMP1 affects autophagocyte formation. We investigated whether endogenous VMP1 interacts with endogenous BECLIN1 in the induction of autophagy in colorectal cancer cells.

Co-immunoprecipitation assays were used to study the interaction of endogenous VMP1 and endogenous BECLIN1 in SW480 cells, as shown in Fig. 4A–D. We observed an increase in VMP1-BECLIN1 complex protein levels in serum-starved or rapamycin-treated cells compared to normal SW480 cells. These findings confirmed the hypothesis that VMP1 binds to BECLIN1 to promote autophagy.

Taken together, our discoveries are: (1) confirmation of VMP1 as a regulator of autophagy in colorectal cancer cell lines; and (2) the first report of a pro-survival role of VMP1-mediated autophagy in colorectal cancer cell lines.

4. Discussion

VMP1 is a promoter of autophagy in many cell types [12], but the role of VMP1 in colorectal cancer is unknown. In this study, we focused on investigating the function of VMP1 in autophagy and apoptosis. Similar to its role in other cell lines, in colorectal cancer cell lines, VMP1 is involved in induction of autophagy; in contrast to pancreatic cell lines, VMP1-dependent autophagy is a pro-survival mechanism. Additionally, our results indicate that VMP1 induces autophagy in colorectal cancer cells by interacting with BECLIN1.

Autophagy is believed to play a role in determining cell fate by degrading old organelles, recycling cellular components and responding to stressful cellular conditions [21]. The relationship between cancer and autophagy has been extensively studied in recent years [22,23]. Our data are consistent with work by Sato et al., who showed that autophagy is activated in colorectal cancers

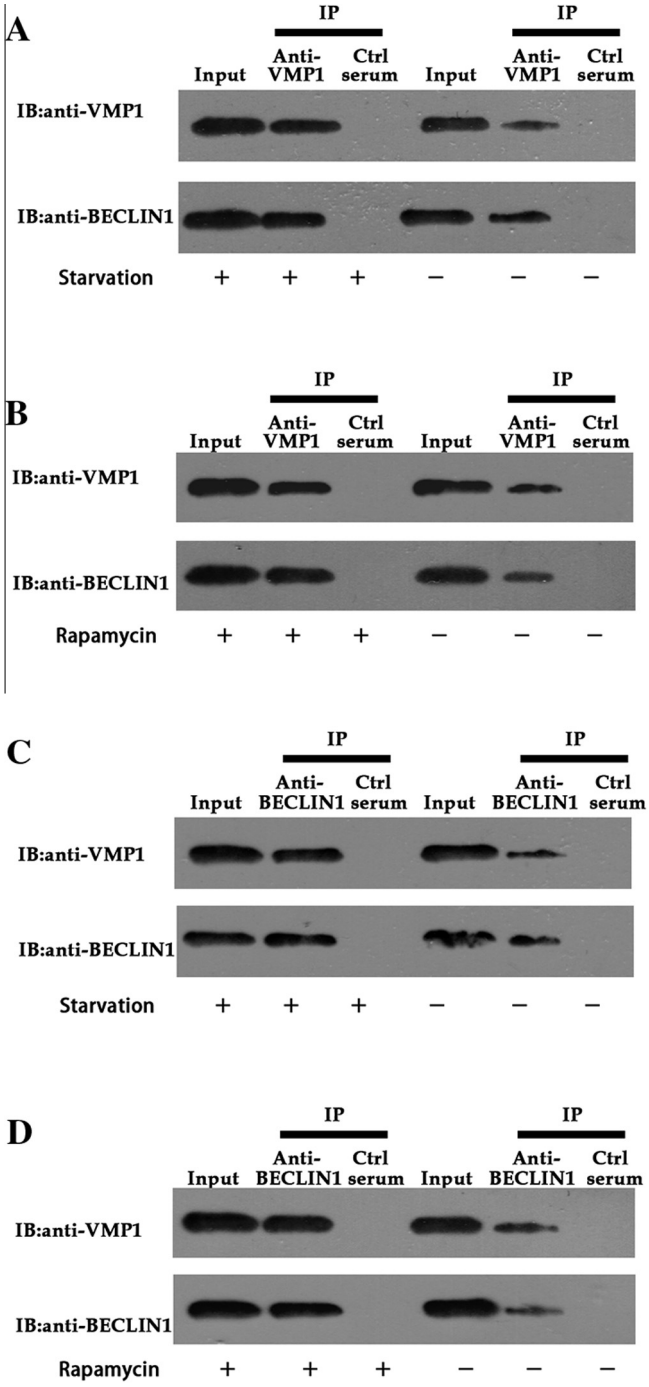

Fig. 4. VMP1 interacts with BECLIN1 in SW480 cells. (A and B) Immunoblots of SW480 cell immunoprecipitates from cells that were serum starved for 12 h (A) or treated with 100 nM rapamycin (B). Cell lysates were immunoprecipitated with anti-VMP-1 antibodies and immunoblotted for VMP1 and BECLIN1. (C and D) Immunoblots of SW480 cell immunoprecipitates from cells that were serum starved for 12 h (C) or treated with 100 nM rapamycin (D). Cell lysates were immunoprecipitated with anti-BECLIN1 antibodies and immunoblotted for VMP1 and BECLIN1. The data are representative of three independent experiments.

in vitro and in vivo and that autophagy could contribute to the survival of cancer cells in their microenvironment [10]. In our study, TEM analysis showed that autophagy was induced when cancer cells were starved.

Previous studies have indicated that VMP1 regulates autophagy in HeLa, PANC-1, MIAPaCa-2, 293T and NIH3T3 cells [12,20], but the function of VMP1 in colorectal cancer cells has not been investigated. Our study demonstrated that starvation induced VMP1 expression in colorectal cancer cells and that knockdown of VMP1 in these cells suppressed autophagy in response to starvation. These results suggest that VMP1 is a crucial factor for the initiation of starvation-induced autophagy in colorectal cancer cell lines.

In this study, we determined that the VMP1-BECLIN1 pathway for activating autophagy that has been described in other cell types is conserved in colorectal cancer cells. BECLIN1 is believed to be a physiologically conserved protein that is believed to be essential for autophagy that interacts with PI3KCIII/Vps34 and other co-factors such as VMP1 to initiate autophagy [24]. BECLIN1 has also been reported to act as a tumor promoter in colon cancer [25], and a previous study has suggested that VMP1 regulates autophagy in HeLa, PANC-1, and AR42J cells by activating BECLIN1 [26]. Our co-immunoprecipitation results demonstrated that VMP1 and BECLIN1 interact when colorectal cancer cells are treated with rapamycin or are serum-starved, suggesting that VMP1 regulates autophagy by interacting with BECLIN1 to induce autophagosome formation. Although this interaction has been described in other cell types, the interaction of VMP1 and BECLIN1 in autophagy pathways in colorectal cancer cells has not been reported previously. Understanding how autophagy is induced in these cell lines could contribute to a better understanding of the mechanisms underlying chemoresistance in colon cancer.

When we induced apoptosis in colorectal cancer cell lines using staurosporine, we did not find a significant reduction in BECLIN1 levels, but we did observe a reduction in VMP1. These results suggest that VMP1 may be an independent factor regulating apoptosis. To confirm that VMP1 protein levels decrease in response to apoptotic stimuli, we exposed several colorectal cancer cell lines to different doses of staurosporine. Our results demonstrate that a decrease in VMP1 protein levels is a common event in colorectal cell lines during apoptosis. This pattern suggests that VMP1 could be an important negative regulator of apoptosis in colorectal cancer.

Previous studies have reported that the VMP1-autophagy pathway promotes apoptosis in pancreatic cancer cells [20]. However, with the evidence of the downregulation of VMP1 during apoptosis, we hypothesized VMP1 might negatively contribute to apoptosis in colorectal cancer cells. SW480 cells were cultured with staurosporine and etoposide, and VMP1 was knocked down with siRNA. Our results demonstrate that SW480 cells were more sensitive to staurosporine- or etoposide-induced apoptosis when VMP1 was knocked down, and VMP1 inhibition resulted in a significant reduction in cell viability under nutrient deprivation. These results suggest that VMP1 plays a pro-survival role in colorectal cell lines.

Our study confirms that VMP1 is an important factor in pathways regulating tumor cell survival in colorectal cell lines. However, there are still questions surrounding the function of VMP1 in the regulation of autophagy and apoptosis in these cells. We revealed one pathway of VMP1-related autophagy, but other pathways may exist in colon cancer cells. Additionally, the mechanism by which VMP1 inhibits apoptosis has not been fully elucidated in these cell lines. There are reports that a dynamic interaction exists between VMP1 and BCL-2 at the mitochondria that could regulate apoptosis [27]. Additionally, it is known that apoptosis can regulate autophagy and that autophagy can inhibit apoptosis [28,29], but the detailed mechanisms concerning the regulation of these two pathways in colon cancer cells are not fully understood. Future studies in animal models with colon-specific overexpression or knockdown of VMP1 are needed to confirm that VMP1 plays a key role in the regulation of autophagy and apoptosis in colon cancer cells in vivo.

In conclusion, this is the first study to examine the role of VMP1 as a regulator of autophagy and apoptosis in colorectal cancer cell lines. We demonstrated that VMP1 is a key pro-survival factor in

colorectal cancer cell lines that acts to promote autophagy by binding to BECLIN1 and inducing the BECLIN1-autophagy program. We also demonstrated that knockdown of VMP1 makes colorectal cancer cells more susceptible to apoptosis, suggesting that VMP1 may be an important negative regulator of apoptotic pathways in these cells. Future studies are needed to explore the detailed mechanisms linking the regulation of autophagy and apoptosis in these cancer cells, and in vivo animal studies are needed to explore the role VMP1 in the pro-survival pathways that contribute to colorectal cancer tumor growth, survival, and chemoresistance.

Disclosure statement

The authors have no conflicts of interest.

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References

[1] A. Grothey, D. Sargent, Overall survival of patients with advanced colorectal cancer correlates with availability of fluorouracil, irinotecan, and oxaliplatin regardless of whether doublet or single-agent therapy is used first line, J. Clin. Oncol. 23 (2005) 9441–9442.
[2] T. Troiani, E. Martinelli, S. Napolitano, F. Morgillo, G. Belli, L. Cioffi, F. Ciardiello, Molecular aspects of resistance to biological and non-biological drugs and strategies to overcome resistance in colorectal cancer, Curr. Med. Chem. (2013). in press.
[3] S. Wu, X. Wang, J. Chen, Y. Chen, Autophagy of cancer stem cells is involved with chemoresistance of colon cancer cells, Biochem. Biophys. Res. Commun. 434 (2013) 898–903.
[4] K. Jain, K.S. Paranandi, S. Sridharan, A. Basu, Autophagy in breast cancer and its implications for therapy, Am. J. Cancer Res. 3 (2013) 251–265.
[5] J.J. Jaboin, M. Hwang, B. Lu, Autophagy in lung cancer, Methods Enzymol. 453 (2009) 287–304.
[6] J. Cui, Z. Gong, H.M. Shen, The role of autophagy in liver cancer: molecular mechanisms and potential therapeutic targets, Biochim. Biophys. Acta 1836 (2013) 15–26.
[7] M.T. Lotze, J. Maranchie, L. Appleman, Inhibiting autophagy: a novel approach for the treatment of renal cell carcinoma, Cancer J. 19 (2013) 341–347.
[8] S. Pandey, Chandravati, Autophagy in cervical cancer: an emerging therapeutic target, Asian Pac. J. Cancer Prev. 13 (2012) 4867–4871.
[9] H.Y. Zheng, X.Y. Zhang, X.F. Wang, B.C. Sun, Autophagy enhances the aggressiveness of human colorectal cancer cells and their ability to adapt to apoptotic stimulus, Cancer Biol. Med. 9 (2012) 105–110.
[10] K. Sato, K. Tsuchihara, S. Fujii, M. Sugiyama, T. Goya, Y. Atomi, T. Ueno, A. Ochiai, H. Esumi, Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation, Cancer Res. 67 (2007) 9677–9684.
[11] N.J. Dusetti, Y. Jiang, M.I. Vaccaro, R. Tomasini, A. Azizi Samir, E.L. Calvo, A. Ropolo, F. Fiedler, G.V. Mallo, J.C. Dagorn, J.L. Iovanna, Cloning and expression of the rat vacuole membrane protein 1 (VMP1), a new gene activated in pancreas with acute pancreatitis, which promotes vacuole formation, Biochem. Biophys. Res. Commun. 290 (2002) 641–649.
[12] A. Ropolo, D. Grasso, R. Pardo, M.L. Sacchetti, C. Archange, A. Lo Re, M. Seux, J. Nowak, C.D. Gonzalez, J.L. Iovanna, M.I. Vaccaro, The pancreatitis-induced vacuole membrane protein 1 triggers autophagy in mammalian cells, J. Biol. Chem. 282 (2007) 37124–37133.
[13] H.D. Xu, D. Wu, J.H. Gu, J.B. Ge, J.C. Wu, R. Han, Z.Q. Liang, Z.H. Qin, The pro-survival role of autophagy depends on Bcl-2 under nutrition stress conditions, PLoS One 8 (2013) e63232.
[14] V. Pagliarini, E. Wirawan, A. Romagnoli, F. Ciccosanti, G. Lisi, S. Lippens, F. Cecconi, G.M. Fimia, P. Vandenabeele, M. Corazzari, M. Piacentini, Proteolysis of Ambra1 during apoptosis has a role in the inhibition of the autophagic pro-survival response, Cell Death Differ. 19 (2012) 1495–1504.
[15] N. Mizushima, Methods for monitoring autophagy using GFP-LC3 transgenic mice, Methods Enzymol. 452 (2009) 13–23.
[16] T.T. Rohn, E. Wirawan, R.J. Brown, J.R. Harris, E. Masliah, P. Vandenabeele, Depletion of Beclin-1 due to proteolytic cleavage by caspases in the Alzheimer's disease brain, Neurobiol. Dis. 43 (2011) 68–78.
[17] G.M. Fimia, M. Corazzari, M. Antonioli, M. Piacentini, Ambra1 at the crossroad between autophagy and cell death, Oncogene 32 (2013) 3311–3318.
[18] E. Wirawan, L. Vande Walle, K. Kersse, S. Cornelis, S. Claerhout, I. Vanoverberghe, R. Roelandt, R. De Rycke, J. Verspurten, W. Declercq, P. Agostinis, T. Vanden Berghe, S. Lippens, P. Vandenabeele, Caspase-mediated cleavage of Beclin-1 inactivates Beclin-1-induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria, Cell Death Dis. 1 (2010) e18.
[19] S. Yousefi, R. Perozzo, I. Schmid, A. Ziemiecki, T. Schaffner, L. Scapozza, T. Brunner, H.U. Simon, Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis, Nat. Cell Biol. 8 (2006) 1124–1132.
[20] R. Pardo, A. Lo Re, C. Archange, A. Ropolo, D.L. Papademetrio, C.D. Gonzalez, E.M. Alvarez, J.L. Iovanna, M.I. Vaccaro, Gemcitabine induces the VMP1-mediated autophagy pathway to promote apoptotic death in human pancreatic cancer cells, Pancreatology 10 (2010) 19–26.
[21] B. Levine, D.J. Klionsky, Development by self-digestion: molecular mechanisms and biological functions of autophagy, Dev. Cell 6 (2004) 463–477.
[22] X.H. Liang, S. Jackson, M. Seaman, K. Brown, B. Kempkes, H. Hibshoosh, B. Levine, Induction of autophagy and inhibition of tumorigenesis by Beclin 1, Nature 402 (1999) 672–676.
[23] R. Mathew, E. White, Autophagy in tumorigenesis and energy metabolism: friend by day, foe by night, Curr. Opin. Genet. Dev. 21 (2011) 113–119.
[24] R. Kang, H.J. Zeh, M.T. Lotze, D. Tang, The Beclin 1 network regulates autophagy and apoptosis, Cell Death Differ. 18 (2011) 571–580.
[25] C.H. Ahn, E.G. Jeong, J.W. Lee, M.S. Kim, S.H. Kim, S.S. Kim, N.J. Yoo, S.H. Lee, Expression of beclin-1, an autophagy-related protein, in gastric and colorectal cancers, APMIS 115 (2007) 1344–1349.
[26] G.M. Fimia, A. Stoykova, A. Romagnoli, L. Giunta, S. Di Bartolomeo, R. Nardacci, M. Corazzari, C. Fuoco, A. Ucar, P. Schwartz, P. Gruss, M. Piacentini, K. Chowdhury, F. Cecconi, Ambra1 regulates autophagy and development of the nervous system, Nature 447 (2007) 1121–1125.
[27] F. Strappazzon, M. Vietri-Rudan, S. Campello, F. Nazio, F. Florenzano, G.M. Fimia, M. Piacentini, B. Levine, F. Cecconi, Mitochondrial BCL-2 inhibits AMBRA1-induced autophagy, EMBO J. 30 (2011) 1195–1208.
[28] M.C. Maiuri, E. Zalckvar, A. Kimchi, G. Kroemer, Self-eating and self-killing: crosstalk between autophagy and apoptosis, Nat. Rev. Mol. Cell Biol. 8 (2007) 741–752.
[29] Y.J. Fan, W.X. Zong, The cellular decision between apoptosis and autophagy, Chin. J. Cancer 32 (2013) 121–129.