Mist1 mediates basal autophagy flux through direct regulation of FIP200 in malignant melanoma cell lines

YIJU LEE1†, WEIFENG YAO2‡, CHUNJUN YANG3, YUNRUI LI4, HAIYANG NI2, LEI WANG2, BIN JI2, YONGGE GU2 and SEN YANG3

1Graduate School, Anhui Medical University, Hefei, Anhui 230032; 2Dermatological Department, Affiliated Hospital of Tianjin Academy of Traditional Chinese Medicine, Tianjin 300120; 3Dermatological Department, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230022; 4School of Basic Medicine, Tianjin Medical University, Tianjin 300070, P.R. China

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Abstract. Malignant melanoma (MM) is a highly aggressive dermatological malignancy with a high tendency to metastasize and a character of refractoriness to conventional treatments after metastasis, which is associated with poor prognosis. For developing more effective therapies, and also for identification of novel biomarkers to predict the behaviour of MM, it is required to fully understand the precise mechanisms underlying melanoma development and progression. The present study compared the expression of muscle, intestine and stomach expression 1 (Mist1) in melanoma cell lines and normal human epidermal melanocytes (NHEM693). It was identified that Mist1 was overexpressed in melanoma cell lines compared with that in NHEM (P<0.05). Mist1 was induced by nutrient starvation in the present study. In eukaryotic cells, autophagy was activated to promote the survival of cells during nutrient starvation. At the same time, the overexpression of Mist1 promoted autophagy flux in human malignant melanoma cells. In addition, loss of Mist1 impaired cellular survival under nutrient starvation conditions. These findings highlighted the importance of Mist1 in cellular autophagy in human malignant melanoma cells. It was also elucidated that FAK family kinase-interacting protein of 200 kDa (FIP200) is a down-stream signaling protein of Mist1. Mist1 regulated cellular autophagy by directly controlling the expression of autophagy gene FIP200. Furthermore, the CCAGCTGC sequence was then uncovered as the binding site of Mist1 in the core region of the FIP200 promoter. The present study suggests that Mist1 has a functional role in promoting melanoma cell autophagy, and that therapeutic strategies directed towards interfering with the action of this gene may be effective for reducing the incidence or development of human melanoma.

Introduction

Melanoma, a highly heterogeneous and aggressive disease, remains to present a remarkable therapeutic challenge. During the past decades, the incidence of malignant melanoma has been increasing, and melanoma is the most common type of fatal malignant melanoma, accounting for 75% of malignant melanoma-associated deaths with a five-year survival rate of <5% (1). Metastasis is one of the characteristics of numerous tumors. Metastasis of melanoma is considered to contribute to the increasing incidence and mortality of malignant melanoma. Even in patients with thin and small primary melanoma, metastasis has also been reported (2). In spite of the tremendous progress made using multimodality therapies including excision, chemotherapy and radiation, the prognosis of malignant melanoma remains poor. Therefore, it is of prime importance to fully understand the mechanism behind the development of malignant melanoma.

Autophagy is an evolutionarily conserved lysosome-dependent cellular degradation pathway, which is integral for homeostasis, differentiation, development and survival. Autophagy is considered to serve an adaptive role in protecting against diverse pathologies, including aging, cancer, neurodegeneration, heart disease and infections (3). In human melanoma cells, inhibition of autophagy resulted in tumor suppression, promotion of apoptosis and obstruction of the cell cycle (4). In a study by Sheen et al (5) using an in vivo tumor xenograft model, the combination of autophagy inhibitor and a leucine-free diet synergistically restrained the growth of melanoma and induced widespread apoptosis of cancer cells.

Correspondence to: Professor Sen Yang, Dermatological Department, The First Affiliated Hospital of Anhui Medical University, 218 Jixi Road, Hefei, Anhui 2300022, P.R. China
E-mail: sen_yangaca@outlook.com

†Contributed equally

Abbreviations: NHEM, normal human epidermal melanocytes; FIP200, FAK family kinase-interacting protein of 200 kDa; mTOR, mammalian target of rapamycin; LC3, microtubule-associated protein 1 light chain 3; GFP, green fluorescent protein

Key words: muscle, intestine and stomach expression 1, FAK family kinase-interacting protein of 200 kDa, autophagy, malignant melanoma cells
Partial autophagy-related protein 5 (Atg5) loss predicted poor overall survival of melanoma patients (6). It was reported that Annexin II receptor reduced the viability of uveal melanoma cells through inducing apoptosis and autophagy and had a protective role in this process (7). Furthermore, overactive autophagy often results in cell death, particularly in cells with deficiency in their apoptotic machinery (3). Hence, autophagy is a highly regulated process, which has a dual role in survival or cell death. In addition, the function of autophagy in determining the outcome of cells is also dependent on the context and cell type. However, the current knowledge on the precise mechanisms of autophagy in the development of malignant melanoma has remained limited. Additional studies may enhance the understanding of the underlying mechanisms of autophagy and supply novel strategies for malignant melanoma treatment.

Muscle, intestine and stomach expression 1 (Mist1), also referred to as DIMMED or class B basic helix-loop-helix (bHLH) protein 8, belongs to the bHLH transcription factors. A variety of serous-secreting exocrine cells were reported to express high levels of Mist1, including lactating mammary acini, pancreatic acinar cells, chief cells of the stomach and salivary gland acinar cells (8,9). Mist1−/− mice are markedly sensitive to pancreatic injury and have a depressed ability to activate the endoplasmic reticulum stress (10). Mist1−/− pancreatic acini exhibited defects in calcium handling and regulated exocytosis. In the salivary glands and pancreas, Mist1 knockout resulted in gene expression alterations and disorganization of the acinar cells (11). However, the role of Mist1 in cancer has remained to be fully elucidated. Mist1 was reported to help gastric stem cells maintain neoplastic growth due to significantly higher expression compared with para-carcinoma tissues. Mist1 was validated to participate in the pathologic process due to significantly higher expression compared with that in para-carcinoma tissues.

A series of Atgs are tightly involved in regulating autophagy. To date, >30 Atgs have been identified in yeast, the majority of which have a mammalian homolog (13). FAK family kinase-interacting protein of 200 kDa (FIP200), also known as RB1 inducible coiled-coil 1, is the homolog of Atg17 in yeast. Deletion of FIP200 leads to an autophagy deficiency (14). FIP200 expression was demonstrated to be strongly associated with disease-free survival and may serve as a novel prognostic marker in breast cancer (15). The present study identified that the expression of Mist1 was significantly higher in melanoma cell lines compared with that in NHEM-a. It was hypothesized that Mist1 is involved in the pathogenesis of human malignant melanoma. Mist1 was validated to participate in the pathologic process due to significantly higher expression compared with that in para-carcinoma tissues.

Plasmid construction and lentiviral transduction. Coding sequences of MIST1 or green fluorescent protein (GFP) combined with microtubule associated protein 1 light chain 3-I (LC3-I) (Beijing Zhongyuan Ltd., Beijing, China) were respectively inserted into a pLVX-IRES plasmid (Beijing Zhongyuan Ltd., Beijing, China). Small hairpin RNA (shRNA) oligonucleotides, shMIST1 (cat. no. 10-295357) or shcon (cat no. 10-295350; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) were inserted onto pLKO.1 plasmids (Addgene; Cambridge, MA, USA). Envelope plasmids (pMD2.G) and packaging plasmids (psPAX2; each from Addgene, Inc.) were co-transfected into 293 cells (American Type Culture Collection, Manassas, VA, USA) with recombinant plasmids using Lipofectamine 3000 (Thermo Fisher Scientific, Inc.). The pLKO.1 plasmid inserted into shcon oligonucleotides was used as scrambled vector. The supernatants containing lentiviruses were collected after 24 h, lentiviruses work as medium of RNA interference and then malignant melanoma cells were infected by lentiviruses. Genes of melanoma cells were subsequently knocked down in the context of lentivirus infection.

Western blot analysis. Mist1-overexpressing cells were treated with 5 nM Baf A1 (cat. no. #1413; Selleck Chemicals, Houston, TX, USA) for 4 h. Prior to analysis, cells were washed with PBS solution 3 times. Cells were lysed using radioimmuno-precipitation assay buffer ( Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The concentration of the protein samples was determined by the bicinchoninic acid method. A total of 60 µg protein per lane was separated by 8-15% SDS-PAGE and then transferred onto a polycryloldene difluoride membrane (GE Healthcare Life Sciences, Little Chalfont, UK). The membrane was blocked using 5% w/v milk at room temperature for 2 h. The primary antibodies were incubated in 4°C overnight. The primary antibodies used in the present study were LC3B (rabbit polyclonal; cat. no. 2775; 1:3,000 dilution; Sangon Biotech, Shanghai, China), mTOR (cat. no. 29833), phospho-mTOR (cat. no. 5536), S6K1 (cat. no. 7004) and phospho-S6K1 (cat. no. 9234), Beclin1 (cat. no. 3495), Atg7 (cat. no. 2631), Atg5 (cat. no. 2630), MIST1/bHLHα15 (cat. no. 14896) (all rabbit polyclonal, used at 1:3,000 dilution; from
Cell Signaling Technologies, Inc., Danvers, MA, USA), FIP200 (goat polyclonal; cat. no. sc-22709; 1:2,000 dilution; Santa Cruz Biotechnology, Dallas, TX, USA) and β-actin (mouse monoclonal; cat. no. A1978; 1:10,000 dilution; Sigma-Aldrich; Merck KGaA) antibodies that were applied in 5% w/v bovine serum albumin (BSA; cat no. A600903; Sangon Biotech Co., Ltd., Shanghai, China). The secondary antibodies used in the present study were anti-rabbit immunoglobulin G (IgG) horseradish peroxidase (HRP)-linked antibody (cat no. 7074; Cell Signaling Technologies, Inc.) and anti-mouse IgG, HRP-linked antibody (cat no. 7076, Cell Signaling Technologies, Inc.). The secondary antibodies (1:3,000 in 5% w/v milk) were incubated in room temperature for 4 h. Bands were visualized using Chemiluminescent HRP substrate (cat. no. P90720; EMD Millipore; Billerica, MA, USA) and images were captured by X-OMAT BT Film (cat. no. 6535876; Carestream Health Inc., Rochester, NY, USA) and blots were quantified using Image J 2.1.4.7 software (National Institutes of Health, Bethesda, MD, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted using TRIZol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and 3 µg was used for RT. RT was performed using a first-strand cDNA synthesis kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. PCR amplification was performed using a SYBR Green kit (Cat no. AQ131, TransGen Biotech Co. Ltd., Beijing, China) on an ABI 7900 system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Thermocycling conditions were as follows: pre-denaturation at 95°C for 3 min, 40 cycles of denaturation at 95°C for 30 sec, annealing at 62°C for 25 sec and extension at 72°C for 25 sec, then a final extension at 72°C for 5 min. Relative gene expression was calculated using the 2^ΔΔCq method (18), with β-actin as the internal control gene. Sequences of the primers were as follows: Mist1 forward, 5'-CCGGATGCAAGCTAATAAC-3' and reverse, 5'-TCGATCTTGGAGACCTTTTT-3'; β-actin forward, 5'-TGGTGGAGCTTCACGACC-3' and reverse, 5'-AGCAGTGGTGGGCCGTACAG-3'. RT-qPCR reactions for each gene were performed in triplicate.

Cell viability assay. Cell viability was examined by an MTT assay using the Roche Cell Proliferation Kit 1 (cat. no. 11465007001; Roche Diagnostics, Basel, Switzerland) according to manufacturer's protocol. All experiments were performed in triplicate. Data were plotted using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA).

Fluorescence microscopy. Human malignant melanoma cells expressing GFP-LC3 were grown on 3.5-cm culture dishes. A fluorescence microscope (Nikon, Tokyo, Japan) was used to capture images of cells expressing GFP-LC3. At least 300 cells from 10 different fields were counted for statistical analysis.

Transmission electron microscopy (TEM). For TEM, human malignant melanoma cells were fixed in a solution of 1% glutaraldehyde, 0.15 M sodium cacodylate, post fixed in and 2.5% OsO₄, and dehydrated in pure ethanol. They were prepared for flat embedding in Epon 812 resin and then observed using a Zeiss CEM 902 electron microscope (Zeiss, Ag, Oberkochen, Germany).

Chromatin immunoprecipitation (ChIP) assay. A total of 10^7 cells were fixed with 1.5% (v/v) methanol-free formaldehyde for 15 min in a 37°C incubator and lysed with SDS lysis buffer. All steps were performed on ice. The lysate subjected to sonication to break the DNA into 200-1,000-bp fragments. Immunoprecipitated DNA by Mist1 antibody (cat no. 14896; 1:100 for overnight at 4°C; Cell Signaling Technologies, Inc., input DNA was analyzed by qPCR according to the aforementioned description and data were presented at input. ChIP analysis was performed using an EZ ChIP kit (cat no. 17-371, EMD Millipore, Billerica, MA, USA) following the manufacturer's protocol with certain modifications according to Fischer et al (19). The experiment was repeated three times.

Reporter assays. The mutated MIST1 promoter and 624 bp DNA fragments were amplified from A375 cells DNA using the overlap extension polymerase chain reaction (OE-PCR) method. The amplified DNA fragments were inserted into the pGL basic plasmid. At 24 h prior to transfection, 1x10^5 cells were seeded into 24-well plates. Plasmid DNA (~800 ng) was complexed with 1 µL Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), and then added to the cells. Renilla reniformis luciferase (pRL-TK) was used as the transfection control. Cells were harvested and lysed 24 h later. Luciferase activity assays were performed using a Dual-Luciferase Reporter Assay System (Promega Corp., Madison, WI, USA) according to a method described by Fischer et al (19). The detections were repeated three times.

Electrophoretic mobility shift assay (EMSA). EMSA was performed using a LightShift EMSA Optimization & Control kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol and according to a method described by Fischer et al (19). The experiment was repeated three times.

Statistical analysis. GraphPad Prism 5 software (GraphPad Software, Inc.) was used to perform the statistical analyses. One-way analysis of variance was performed for comparison analysis. Following this, Dunnnett's test was used for pairwise comparisons of multiple treatment groups. Significant differences were evaluated using Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Human malignant melanoma cells highly express Mist1. To evaluate whether Mist1 is expressed in different malignant or healthy skin cell lines, NHEM693 and two malignant melanoma cell lines, A375 and Mv3, were assessed in this study. Mist1 expression was detected at the protein level using western blot analysis and at the mRNA level using RT-qPCR analysis. The expression of Mist1 in the malignant melanoma cell lines was markedly higher than that in NHEM693, as indicated by western blot analysis (Fig. 1A), and the transcription levels of the Mist1 gene were also significantly higher in malignant melanoma cells (P<0.01 vs. NHEM693; Fig. 1B). To confirm that the Mist1 gene is involved in the pathogenesis of human malignant
melanoma, biopsies from 26 malignant melanoma patients and para-carcinoma tissues were subjected to RT-qPCR. Mist1 was validated to be associated with malignant melanoma due to being significantly higher expressed in malignant melanoma tissues than in para-carcinoma tissues ($P<0.01$; Fig. 1C).

The mammalian target of rapamycin (mTOR) pathway is involved in the autophagic process induced by Mist1 in human malignant melanoma cells. Serum depletion is known to induce marked autophagy. Of note, serum starvation of malignant melanoma cell lines increased Mist1 expression in the present study (Fig. 2A). To evidence the link between Mist1 and autophagy, Mist1 was overexpressed in malignant melanoma cell lines, which resulted in an increase in the levels of LC3-II, the autophagosome-associated lipidated form of LC3, Atg5 and Atg7, the necessary factor for autophagy initiation, and a decrease in p62, an autophagic substrate (Fig. 2B). Furthermore, GFP-LC3, a green fluorescent autophagy reporter...
protein, demonstrated subcellular localization and serum starvation as a positive control. Starvation treatment induced significant GFP-LC3 puncta and GFP-LC3 puncta indicated the formation of autophagosomes. Overexpression of Mist1 increased formation of GFP-LC3 puncta (Fig. 2C) and the average number of GFP-LC3 dots in Mist1 overexpressing cells was significantly increased compared with that of control cells (P<0.01; Fig. 2D). Subsequently, bafilomycin A1 (Baf A1) was applied to block the fusion between autophagosomes and lysosomes in Mist1-overexpressing cells. Further accumulation of LC3-II was observed, and p62 increased under these conditions (Fig. 2E). Furthermore, Mist1-overexpressing cells displayed cytoplasmic accumulation of autophagosomes, as determined by TEM (Fig. 2F). The mTOR pathway is the classic regulator of nutrient stimuli and active mTOR inhibits autophagy. Next, the mTOR pathway was investigated by immunoblotting using respective antibodies against mTOR, phospho-mTOR, S6K1 and phospho-S6K1 in Mist1-overexpressing cells. The results indicated that the inactivation of the mTOR pathway was involved in the autophagic process induced by Mist1 (Fig. 2G).

**Loss of Mist1 impairs cellular survival under nutrient starvation conditions.** The aforementioned results suggested that Mist1 induces autophagy. To further evaluate whether Mist1 participates in the regulation of autophagy, lentiviral plasmids expressing shRNA targeting different regions of Mist1 mRNA designed and constructed. The silencing efficiency of two different shRNAs against Mist1 was evaluated by RT-qPCR and western blot analysis (Fig. 3A). No. 1 shRNA was used to knock down Mist1 in the subsequent experiment. Next, autophagy-associated biomarkers including LC3-II, Atg5, Atg7 and p62 were assessed in Mist1-knockdown malignant melanoma cells treated with HBSS (Fig. 3B). The results indicated that loss of Mist1 impaired autophagy in A375 human malignant melanoma cells. These results were further confirmed by fluorescent microscopy (Fig. 3C) and TEM (Fig. 3D). These cell images indicated that silencing of Mist1 resulted in suppression of autophagy. Autophagy is an important cellular survival mechanism under starvation culture, and long-term starvation undoubtedly results in cell death. To investigate cellular survival after silencing of Mist1, a cell viability assay was performed. As expected, silencing of Mist1 significantly impaired cellular survival of A375 cells (Fig. 3E). In addition, similar results were obtained with another cell line, MV3 (data not shown). Taken together, these results indicated that Mist1 is critical for basal cellular autophagy and activates autophagy via certain mechanisms.

**FIP200 is a direct regulatory target of Mist1 in human malignant melanoma cells.** As predicted by the JASPAR database (http://jaspar.genereg.net), Mist1 may bind to a sequence in the promoter region of FIP200. Of note, a positive association between Mist1 and FIP200 levels was observed in A375 and MV3 cells (Fig. 4A and B). Furthermore, downregulation of FIP200 restrained autophagy activated by Mist1 (Fig. 4C). Based on these results, it was hypothesized that FIP200 may be a target gene of Mist1 in human malignant melanoma cells. Next, the interaction status of Mist1 in genomic loci of FIP200 was examined using a ChIP assay. Bound DNA fragments were eluted and analyzed by subsequent qPCR. The putative Mist1-binding site from JASPAR database was indicated as site 7. The occupancy of a region by Mist1 was examined via
Figure 4. Mist1 controls the transcription of FIP200 and the CCAGCTGC motif is required for Mist1 binding to the FIP200 promoter. (A and B) Immunoblot analysis of Mist1 and FIP200 in whole-cell lysates from A375 and MV3 cells (A) after knockdown of Mist1 and (B) after vector-mediated overexpression of Mist1, with β-actin as a loading control. (C) In A375 cells with vector-mediated overexpression of Mist1, FIP200 was silenced or not. The effect on autophagy-associated proteins was examined by immunoblot analysis, with β-actin as a loading control. (D) Chromatin immunoprecipitation analysis of Mist1 binding to the FIP200 promoter region. The data were presented as the fold of template enrichment in immunoprecipitates of anti-Mist1 relative to those of control immunoglobulin G. **P<0.01 vs. control. (E) A375 cells were transfected with luciferase reporter plasmids containing the core promoter of FIP200 or not. Bars indicate the mean relative luciferase units as arbitrary light units. *P<0.001. (F) The putative Mist1 binding motif from the JASPAR database (top), and luciferase reporter assay of the mutagenesis experiment within the putative Mist1 binding site (bottom). Sites of mutation are indicated in red letters. *P<0.001. (G) An electrophoretic mobility shift assay demonstrated a mobility shift of a probe with Mist1 consensus sites, with a supershift following anti-Mist1 treatment. All quantitative data are expressed as the mean ± standard deviation of at least 3 independent experiments. Mist1, muscle, intestine and stomach expression 1; Mut, mutated; WT, wild-type; LC3, microtubule-associated protein 1 light chain 3; shFIP200, small hairpin RNA targeting FAK family kinase-interacting protein of 200 kDa; TSS, transcription start site.
a ChIP-qPCR assay and data indicated that site 7 interacted with Mist1 (Fig. 4D). In addition, a luciferase reporter assay demonstrated that the core promoter region covered the -616 to +26-bp fragment (Fig. 4E). Of note, the CCAGCTGC sequence was uncovered to be the binding site of Mist1 in the core region of the FIP200 promoter, and a mutagenesis experiment, in which different bases within the putative Mist1 binding site were mutated, led to the identification of the accurate binding site of Mist1 in the FIP200 promoter (Fig. 4F). In addition, a clear supershift in an EMSA indicated the direct interaction of Mist1 with this sequence as expected (Fig. 4G). These results provided solid evidence for the hypothesis that Mist1 directly regulates FIP200 transcription by binding to the core promoter region of FIP200.

Discussion

Melanoma, a highly heterogeneous and aggressive disease, remains to present a remarkable therapeutic challenge. During the past decades, the incidence of malignant melanoma has been increasing, and melanoma is the most common type of fatal malignant melanoma, accounting for ~75% of malignant melanoma-associated deaths with a five-year survival probability of <5% (1). In spite of tremendous progress made in multimodality therapies, including excision, chemotheraphy and radiation therapy, the prognosis of malignant melanoma remains poor. Therefore, it is of prime importance to fully understand the mechanism behind the development of malignant melanoma.

Differential expression of genes in cancer has attracted increasing attention. In the present study, the role of bHLH transcription factor Mist1 was evaluated in human malignant melanoma cells. A variety of serous-secreting exocrine cells have been reported to express high levels of Mist1, including lactating mammary acini, pancreatic acinar cells, chief cells of the stomach and salivary gland acinar cells (8,9). In the present study, it was observed that levels of Mist1 were significantly higher in malignant melanoma cells lines compared with those in NHEM693. The cancer biopsy specimens from 26 malignant melanoma patients and their para-carcinoma tissues were subjected to RT-qPCR analysis, demonstrating a similar trend. As in malignant melanoma tissues and cell lines, Mist1 was significantly higher expressed than in para-carcinoma tissues and NHEM693, respectively, it was validated to be associated with malignant melanoma.

Autophagy is a catabolic process in which cytoplasmic constituents are transferred to the lysosome for digestion. Increasing evidence has suggested that the autophagic process is involved in adaptation to starvation, as well as aging, cell-cycle regulation and cancer development (3). In the present study, autophagy was measured by examining the levels of LC3-II, the autophagosome-associated lipidated form of LC3, Atg5 and Atg7, the necessary factor for autophagy initiation, and p62, an autophagic substrate. The results indicated that serum starvation increased Mist1 expression. Malignant melanoma cells with vector mediated overexpression of Mist1 demonstrated cytoplasmic accumulation of autophagosomes, as determined by TEM. The mTOR pathway is the classic regulator of nutrient stimuli, and active mTOR inhibits autophagy, mTOR complex 1 is a major growth regulator, which modulates cellular anabolism and cellular catabolism with the availability of critical nutrients such as amino acids (20). The present results indicated that deactivation of the mTOR pathway was involved in the autophagic process induced by Mist1.

To date, >30 Atgs have been confirmed in yeast, including Atg1-10, 12-14, 16-18, 29 and 31, most of which are evolutionarily conserved in eukaryotes and are essential for autophagosome formation (21,22). In the present study, it was indicated that FIP200, a homolog of Agt17 in yeast, was the target gene of transcription factor Mist1 in human malignant melanoma cells. Overexpression of Mist1 induced FIP200, while downregulation of Mist1 decreased the expression of FIP200. In turn, knockdown of FIP200 restrained Mist1-induced autophagy. The interaction between Mist1 and genomic loci of FIP200 was then assessed using a ChIP assay. The occupancy of segments of the prospective binding region by Mist1 was examined via a ChIP-qPCR assay and the putative Mist1-binding site from the JASPAR database was confirmed. In addition, a luciferase reporter assay provided evidence that the core promoter region of FIP200 targeted by Mist1 covered the -616 to +26-bp fragment. Of note, the CCAGCTGC sequence was uncovered as the binding site of Mist1 in the core region of the FIP200 promoter. A point mutation of the CCAGCTGC sequence inhibited the ability of Mist1 to interact with the FIP200 promoter sequence in the reporter plasmid. During the autophagosome formation process, Atg9A and the ULK1-Atg13-FIP200-Atg101 complex are independently recruited to the site of autophagosome formation, and both are essential for the recruitment of Beclin1-Atg14L-Vps15-Vps34 complex. The activity of type III phosphatidylinositol-3 kinase is essential for further recruitment of Atg12-Atg5-Atg16L1 and Atg2-WIPI complexes (23,24). Finally, LC3 is recruited dependent on an Atg12-Atg5-Atg16L1 (25). Therefore, Mist1 may take an important role in the initial period of autophagy by controlling the expression of FIP200. However, a single increase of FIP200 is inadequate for activation of autophagy. Therefore, Mist1 may promote further processes to activate autophagy, which requires additional study. MIST1 is likely to have multiple target genes, and the extent of the involvement of Mist1 in regulating the autophagic pathway deserves further study.

In conclusion, the results of the present study comprehensively proved the hypothesis that Mist1 contributes to autophagy through directly regulating FIP200 in human malignant melanoma cells. The novel information gained from the present study significantly contributes to elucidating the molecular mechanism underlying the regulation of autophagy through Mist1. However, this study is still of preliminary nature. Further study is required to confirm the function of Mist1 in the regulation of FIP200 and autophagy in human malignant melanoma and other malignant cancers in vivo. The regulation of autophagy by Mist1 via targeting FIP200 may provide a novel therapeutic approach to develop targeted therapies for melanoma.

References