Biochemical and Biophysical Research Communications 430 (2013) 810–815

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Stabilization of MAPO1 by specific binding with folliculin and AMP-activated protein kinase in $O^6$-methylguanine-induced apoptosis

Shiori Sano [a,b], Ryuji Sakagami [a], Mutsuo Sekiguchi [c], Masumi Hidaka [b,*]

[a] Department of Odontology, Fukuoka Dental College, Fukuoka 814-0193, Japan
[b] Department of Physiological Science and Molecular Biology, Fukuoka Dental College, Fukuoka 814-0193, Japan
[c] Advanced Science Research Center, Fukuoka Dental College, Fukuoka 814-0193, Japan

ARTICLE INFO

*Article history:*
Received 13 November 2012
Available online 29 November 2012

*Keywords:*
AMPK
Apoptosis
Folliculin/BHD
MAPO1/FNIP2/FNIPL
$O^6$-methylguanine

ABSTRACT

When DNA is damaged by alkylating agents, apoptosis is induced to exclude cells carrying DNA lesions in order to prevent mutations and cancer. MAPO1, identified as a component involved in the induction of apoptosis, interacts with AMP-activated protein kinase (AMPK) and folliculin (FLCN). We herein report that MAPO1 is stabilized during the course of apoptosis, triggered by alkylation-induced $O^6$-methylguanine in DNA. An immunoblotting analysis revealed that the amount of MAPO1 increased gradually after treatment with *N*-methyl-*N*-nitrosourea (MNU), although the level of mRNA for *MAPO1* was unchanged. When cells were exposed to a proteasome inhibitor, MG132, the MAPO1 level significantly increased. On the other hand, application of a protein synthesis inhibitor, cycloheximide, caused a decrease in the MAPO1 content, implying that proteasome-mediated degradation is involved. In *FLCN*-knockdown cells, the MAPO1 level decreased, and no increases occurred even after MNU treatment. In contrast, stabilization of MAPO1 occurred in *AMPK*α-knockdown cells even without MNU treatment. While MAPO1 retains its ability to stably bind to FLCN, it dissociates gradually from AMPK after exposure to MNU. It seems that the proapoptotic function of MAPO1 may be regulated by AMPK and FLCN through stabilization of MAPO1 itself.



## 1. Introduction

Among various modified bases in DNA produced by treatment with alkylating agents, $O^6$-methylguanine is of particular importance since this modified base can pair with thymine as well as cytosine during DNA replication, thus inducing both killing and mutation [1,2]. Organisms possess an enzyme, $O^6$-methylguanine-DNA methyltransferase (MGMT), that repairs this DNA lesion [3]. Mice, in which the *Mgmt* gene encoding the enzyme was disrupted, exhibited hypersensitivity to both killing and tumorigenic actions of simple alkylating agents, such as *N*-methyl-*N*-nitrosourea (MNU), and drugs with alkylating capacity [4–8]. These dual effects of alkylating agents can be dissociated by introducing an additional mutation in the *Mlh1* gene, which encodes a protein functioning at an initial step in apoptosis, to the mice. Mice with mutations in both the *Mgmt* and *Mlh1* genes are as resistant to MNU as are wild-type mice in terms of survival, but develop numerous tumors after receiving MNU. In contrast to $Mgmt^{-/-}$ mice, which exhibit hypocellular bone marrow and considerable decreases in the size of the thymus after MNU administration, no conspicuous changes are found in $Mgmt^{-/-}Mlh1^{-/-}$ mice treated in the same manner [9]. Thus, the tumorigenic effects of $O^6$-methylguanine are protected by both MGMT-mediated DNA repair and MLH1-dependent apoptosis.

The mismatch repair (MMR) protein complex, composed of MSH2, MSH6, MLH1 and PMS2, recognizes an $O^6$-methylguanine and thymine mispair, formed during DNA replication after treatment of alkylating agents, and induces apoptosis [10–12]. The formation of this complex further induces the activation of cell cycle checkpoints and the release of cytochrome C from the mitochondria as well as the activation of Apaf-1 and caspase-3 [13,14]. By using a gene-trap mutagenesis, we identified *Mapo1* ($O^6$-methylguanine-induced apoptosis 1) as a new gene that functions at a certain step in apoptosis triggered by MNU [15]. Mouse-derived KH101 cells, which carry an insertional mutation in one of the *Mapo1* alleles, are unable to induce mitochondrial membrane depolarization as well as caspase-3 activation after treatment with MNU. It is noteworthy that the mutant frequency of KH101 cells is significantly elevated after MNU treatment. Subsequently, MAPO1 was found to be identical to a protein named FNIP2 or FNIPL, having the capacity to interact with folliculin [16,17]. Folliculin is a tumor suppressor protein encoded by the *FLCN* gene, and mutations in the *FLCN* gene are found in patients with Birt–Hogg–Dubé (BHD) syndrome,

* Corresponding author. Address: Department of Physiological Science and Molecular Biology, Fukuoka Dental College, 2-15-1 Tamura, Sawara-ku, Fukuoka 814-0193, Japan. Fax: +81 92 801 0685.
*E-mail address:* hidaka@college.fdcnet.ac.jp (M. Hidaka).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.11.064

which is characterized by the development of hair follicle hamartomas and lung cysts and an increased risk of renal neoplasia [18,19]. *Flcn* heterozygous knockout mice develop kidney cysts and tumors as they age, while *Flcn* homozygous null mice display early embryonic lethality [20,21]. In addition to a binding capacity for folliculin, MAPO1 has the capacity to bind to 5′-AMP-activated protein kinase (AMPK), which is composed of AMPKα, β and γ subunits and is a cellular energy sensor that negatively regulates cell growth and proliferation [22,23]. By interacting with folliculin and AMPK, MAPO1 might execute its function in the course of induction of apoptosis. This notion was supported by the finding that knockdown of the *Flcn* and *Ampkα* genes by specific siRNAs significantly suppresses an apoptotic response to MNU. There are evidences that AMPK is activated during the process of $O^6$-methylguanine-induced apoptosis and that this activation is dependent on MAPO1 and folliculin [24]. Furthermore, it has been reported that *Flcn*$^{-/-}$ embryonic stem cells are unable to induce apoptosis, most likely due to their impaired expression of one of the Bcl-2 family of proteins, Bim [25].

It is important to elucidate how MAPO1 interacts with folliculin and AMPK during apoptosis and how this process is regulated. We herein report that the amount of MAPO1 increases following stabilization of the protein during the course of apoptosis triggered by MNU. We obtained evidence to show that stabilization of MAPO1 is regulated through its specific interaction with folliculin and AMPK.

## 2. Materials and methods

### 2.1. Cell lines and cell culture

HeLa MR, which is defective in $O^6$-methylguanine-DNA methyltransferase activity, was used [11]. From HeLa MR, the *MAPO1*-knockdown cell lines (M-KD1 and M-KD2) were established by expressing specific miRNAs, the sequences of which are 5′-TGCTGTGCACACTCCATTTATCCGTGGTTTTGGCCACTGACTGACCACG GATATGGAGTGTGCA-3′ and 5′-TGCTGTTCTGAGGACAGCAAACATT CGTTTTGGCCACTGACTGACGAATGTTTTGTCCTCAGAA-3′, respectively, followed by selection with 5 μg/ml of blasticidin. The miRNA expression vectors were constructed using the BLOCK-iT Pol II miR RNAi Expression Vector Kit with EmGFP (Invitrogen), according to the manufacturer's instruction manual. The HeLa MR stably expressing Flag-tagged MAPO1 was constructed by introducing p3xFLAG-CMV10 (Sigma–Aldrich) containing human *MAPO1* cDNA, followed by selection with 600 μg/ml of G418. All cells were cultivated in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 10% fetal bovine serum at 37 °C in 5% $CO_2$.

### 2.2. Chemicals

*N*-Methyl-*N*-nitrosourea (MNU), Cycloheximide and MG132 were purchased from Sigma–Aldrich, Nacalai tesque and Wako Pure Chemical Industries Ltd., respectively.

### 2.3. Flow cytometric analysis

For the sub-$G_1$ population assay, cells were washed with PBS and suspended in 400 μl of PBS containing 0.1% Triton X-100, 25 μg/ml of propidium iodide and 0.1 mg/ml of RNase A. The samples were analyzed using a FACS Calibur flow cytometer (Becton Dickinson) with 10,000 events per determination.

### 2.4. Quantitative real-time PCR analysis

Total RNA was prepared from cells with the RNeasy Mini Kit (QIAGEN) and used to synthesize cDNAs with PrimeScript Reverse Transcriptase (Takara Bio Inc.). Real-time PCR was performed with the 7500 Real-Time PCR System (Applied Biosystems) using SYBR Premix Ex Taq II (Takara Bio Inc.). The PCR primers for the *MAPO1* gene, 5′-TGTCCAGTTTGCTTCAGTCCATT-3′ and 5′-CAGAGGCAGG TCGTTGGATTC-3′, and for the *GAPDH* gene as a reference, 5′-GCACCGTCAAGGCTGAGAAC-3′ and 5′-ATGGTGGTGAAGACGCC AGT-3′, were purchased from Takara Bio Inc.

### 2.5. Immunoprecipitation and immunoblotting

For immunoprecipitation, Flag-tagged MAPO1-expressing cells were lysed with buffer A (50 mM Tris/HCl (pH 8.0), 150 mM NaCl, 0.2% Triton X-100, 1 mM EDTA) containing protease inhibitors (Roche). To precipitate the Flag-tagged MAPO1, 10 μl of anti-FLAG M2-agarose (Sigma–Aldrich) were added to the extract and incubated for 4 h at 4 °C. After extensive washing of the beads with buffer A, the proteins bound to the beads were eluted in 50 μl of 2xSDS–PAGE sample buffer (100 mM Tris/HCl (pH 6.8), 4% SDS, 20% glycerol, 200 mM DTT, 0.002% bromophenol blue).

For the immunoblotting analyses, immunoprecipitated materials or whole cell extracts prepared from lysis of the cells with 2xSDS–PAGE sample buffer were subjected to SDS–PAGE and electroblotted onto a PVDF membrane (Bio-Rad). Detection was performed using an ECL Prime or Advance Western blotting detection kit (GE Healthcare). The primary antibodies used were: anti-FNIP2 (Sigma–Aldrich) for MAPO1, anti-FLCN (Protein Tech Group, Inc), anti-AMPKα (Cell signaling), anti-GAPDH (Cell signaling), and anti-FLAG M2 (Sigma–Aldrich).

### 2.6. siRNA transfection

Stealth RNAi for the *FLCN* gene (siFLCN), 5′-ACACCUC CUUUGCCUGGCUCCUGAA-3′, was purchased from Invitrogen. AMPKα1/2 siRNA (sc-45312) was obtained from Santa Cruz. After culturing $4 \times 10^5$ cells in a 6-well plate for one day, the cells were transfected with 100 nM siRNA using the Lipofectamine RNAiMAX reagent (Invitrogen) according to the manufacturer's protocol. For the control transfection, Stealth RNAi Negative Control Medium GC Duplex (Invitrogen) was used.

### 2.7. Statistics

All *P*-values were generated using two-tailed Student's *t*-tests.

## 3. Results

### 3.1. Stabilization of MAPO1 during apoptosis triggered by MNU

Two types of *MAPO1*-knockdown cell lines, M-KD1 and M-KD2, were obtained by introducing miRNAs with different *MAPO1* sequences into human HeLa MR, which is defective in $O^6$-methylguanine-DNA methyltransferase activity. The quantitative real-time PCR analysis revealed that the expression level of the *MAPO1* gene in M-KD1 and M-KD2 cells is 37.1% and 24.2% of that of HeLa MR, respectively. These cells were treated with or without 0.4 mM *N*-methyl-*N*-nitrosourea (MNU), and were subjected to flow cytometric analysis. As shown in Fig. 1A, production of the sub-$G_1$ population of cells 80 h after MNU treatment was significantly suppressed in the *MAPO1*-knockdown cells, as compared to HeLa MR. These results indicate that MAPO1 is involved in MNU-induced apoptosis in human cells.

To measure the expression level of MAPO1 in the course of apoptosis, HeLa MR cells were treated with 1 mM MNU for 1 h and then collected at various times after treatment. The cells not exposed to MNU were similarly treated. The prepared cell extracts

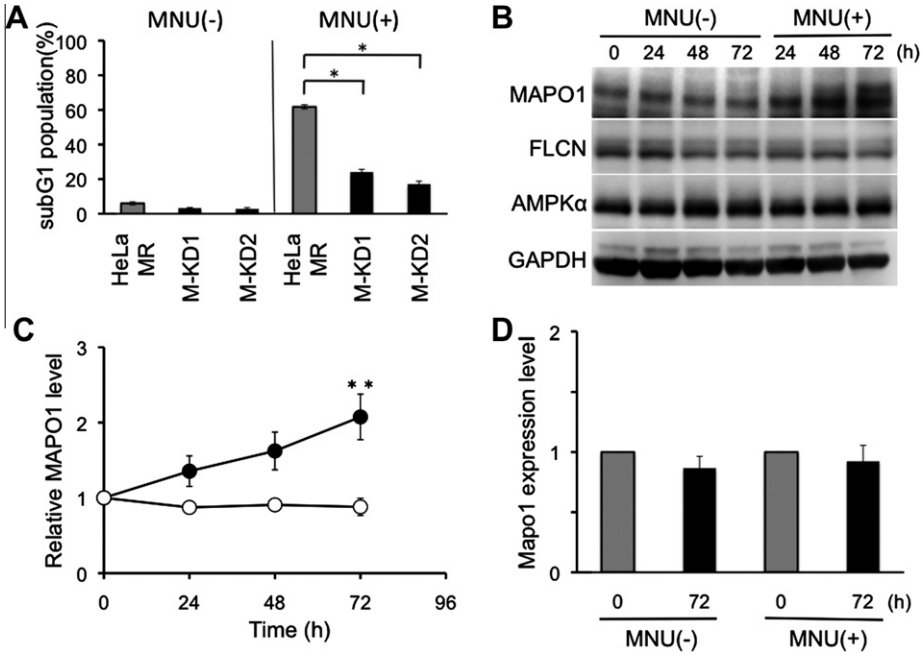


**Fig. 1.** The change in the amount of MAPO1 during MNU-induced apoptosis. (A) The sub-$G_1$ population of cells after MNU treatment. HeLa MR and *MAPO1*-knockdown cells were treated with or without 0.4 mM MNU for 1 h and incubated for 80 h. The cells were harvested and then subjected to a flow cytometric analysis. The mean values of the sub-$G_1$ cell population and the standard deviations obtained from more than three independent experiments are presented. $^{*}P < 0.01$. (B) The immunoblotting analysis. HeLa MR cells were treated with or without 1 mM MNU for 1 h and then incubated for 0, 24, 48 or 72 h in a drug-free medium. The whole cell extracts were prepared at the indicated times and subjected to an immunoblotting analysis using antibodies against MAPO1, FLCN, AMPKα and GAPDH. (C) The change in the level of MAPO1 after MNU treatment. The data were taken from the analysis shown in (B). Open circles, without MNU treatment; closed circles, with MNU treatment. $^{**}P < 0.05$ when comparing the relative intensity of MAPO1 at 72 h with and without MNU treatment. (D) The level of mRNA for *MAPO1*. HeLa MR cells treated with or without 1 mM MNU were harvested at 0 and 72 h of postincubation, and the expression levels of the *MAPO1* gene were measured using quantitative real-time PCR.

were subjected to an immunoblotting analysis using specific antibodies against MAPO1 as well as FLCN and AMPKα. As shown in Fig. 1B and C, the level of MAPO1 increased gradually after MNU treatment and reached approximately 2.1-fold at 72 h. On the other hand, the levels of FLCN and AMPKα proteins were constant after MNU treatment (Fig. 1B). No such increases in the level of MAPO1 were observed in the *MLH1*-knockdown cells, which lost the ability to induce apoptosis (Supplementary Fig. 1). The quantitative real-time PCR analysis revealed that the mRNA expression level of the *MAPO1* gene was unchanged at 72 h, even after treatment with MNU (Fig. 1D), indicating that increases in the level of MAPO1 are not mediated by transcriptional regulation.

To determine whether the amount of MAPO1 proteins is regulated at the post-translational level, HeLa MR cells that produce Flag-tagged MAPO1 under the control of the CMV promoter were treated with a proteasome inhibitor, MG132. As shown in Fig. 2A and B, the amount of MAPO1 significantly increased 6 and 12 h after treatment with MG132. This was also the case for p53, which is constitutively degraded in HeLa MR by papilloma virus E6 in an ubiquitin/proteasome system-dependent manner [26]. In contrast, the amounts of FLCN and AMPKα were unchanged by the drug treatment. These results are shown in Fig. 2A. When the cells were treated with a protein synthesis inhibitor, cycloheximide, the level of MAPO1 protein decreased dramatically (Fig. 2C and D). Under these conditions, the amounts of FLCN and AMPKα were not significantly changed (Fig. 2C). It seems that MAPO1 proteins may be stabilized when cells undertake apoptosis.

### 3.2. The effects of FLCN and AMPK on the stability of MAPO1

Since MAPO1 is known to interact with FLCN and AMPK, it is plausible that the interacting proteins might have some effects on the stability of MAPO1 proteins. To investigate this problem, siRNAs specific for the *FLCN* and *AMPKα* genes were introduced into HeLa MR cells. As shown in Fig. 3A, siRNAs for *FLCN* and *siAMPKα* effectively suppressed the expression of each of the genes. To investigate the effects of *FLCN*- and *AMPKα*-knockdown on the stability of MAPO1 during apoptosis, the cells were treated with or without 1 mM MNU for 1 h and the cell extracts were prepared 72 h after drug treatment. The materials derived from the three types of cells were subjected to an immunoblotting analysis to measure the amounts of MAPO1 proteins (Fig. 3B). In the *FLCN*-knockdown cells, the amount of MAPO1 decreased to 41% of that in the control cells transfected with siCONT, and there were no significant responses to MNU treatment. In the *AMPKα*-knockdown cells, on the other hand, the level of MAPO1 increased more than two times that observed in the siCONT-transfected cells and increased further after exposure to MNU. These results suggest that FLCN may act positively on the stabilization of MAPO1, whereas AMPK might be involved in the degradation of MAPO1. To examine whether the kinase activity of AMPK is involved in the process of degradation of MAPO1, cells treated with or without 1 mM MNU for 1 h were incubated in a medium containing a specific inhibitor of AMPK, compound C, for 72 h. An immunoblotting analysis revealed that the level of MAPO1 significantly increased after the administration of compound C in the MNU-untreated cells, whereas no increases in the MAPO1 level were observed, after treatment with MNU (Supplementary Fig. 2). It is likely that the kinase activity of AMPK is involved in the degradation of MAPO1.

### 3.3. A change in the interaction of MAPO1 with AMPK and FLCN following MNU treatment

To follow the patterns of physical interaction of MAPO1 with FLCN and AMPK during the process of apoptosis induction, HeLa MR cells expressing Flag-tagged MAPO1 were treated with 1 mM

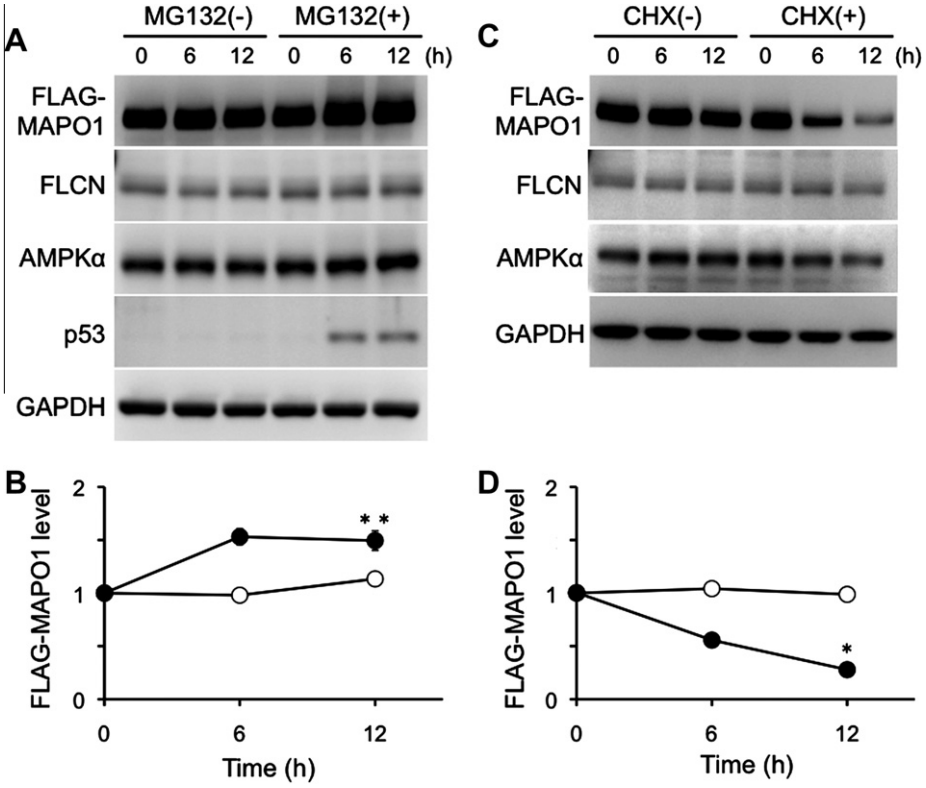


**Fig. 2.** Proteasome-dependent degradation of MAPO1. (A) The protein levels in the MG132-treated cells. HeLa MR cells expressing Flag-tagged MAPO1 were incubated in a medium with or without 100 μM MG132. At times of 0, 6 and 12 h, the cells were harvested, and the prepared extracts were subjected to an immunoblotting analysis using antibodies that recognize Flag-tag, FLCN, AMPKα, p53 and GAPDH, respectively. (B) Changes in the MAPO1 levels after MG132 treatment. Open circles, MG132(−); closed circles, MG132(+). $^{**}P < 0.05$ when comparing the relative intensity of Flag-MAPO1 at 12 h with and without MG132 treatment. (C) The protein levels in the CHX-treated cells. HeLa MR cells expressing Flag-tagged MAPO1 were incubated in a medium with or without 20 μM cycloheximide (CHX). An immunoblotting analysis using antibodies that recognize Flag-tag, FLCN, AMPKα and GAPDH was performed as described in (A). (D) The relative intensities of MAPO1 after CHX treatment. Open circles, CHX(−); closed circles, CHX(+). $^{*}P < 0.01$ when comparing the relative intensity of Flag-MAPO1 at 12 h with and without CHX treatment.

MNU for 1 h. Whole cell extracts were prepared at 0, 24, 48 and 72 h of incubation and applied to immunoprecipitation using an anti-Flag antibody conjugated to agarose beads followed by an immunoblotting analysis (Fig. 4). At time 0, MAPO1 was evidently associated with both FLCN and AMPKα, as previously reported [16,17,24]. A stable interaction with FLCN was retained throughout the time of treatment with MNU. In contrast, the interaction of MAPO1 with AMPKα gradually decreased and was hardly detected 72 h following MNU exposure. This dissociation of AMPK from MAPO1 might play a role in the execution of apoptosis.

## 4. Discussion

*Mapo1* was identified as a gene functioning at a certain step in apoptosis triggered by MNU. In murine cells defective in the *Mapo1* gene, mitochondrial membrane depolarization and activation of caspase-3, both of which are hallmarks for the induction of apoptosis, were not observed after treatment with MNU [24]. In the present study, by using human-derived cell lines we confirmed the existence of this proapoptotic function of MAPO1 and further established the role of MAPO1 in execusion of apoptosis. *MAPO1*-knockdown cells constructed from MGMT-defective HeLa MR exhibited an increased level of resistance to MNU and produced a smaller transitions to sub-$G_1$ populations, as compared to the parental cells. An immunoblotting analysis revealed that the amount of MAPO1 increased in cells after treatment with MNU. Since this increase does not occur in *MLH1*-knockdown cells, it is implied that the increase of MAPO1 is associated with the process of MMR-dependent apoptosis. It was revealed that the amount of MAPO1 is regulated at the post-translational level, since the MAPO1 level increased after treatment with a proteasome inhibitor, MG132, and decreased following exposure to a protein synthesis inhibitor, cycloheximide. Under ordinary conditions, the amount of MAPO1 is maintained at a certain low level, due to its constitutive synthesis and degradation by the proteasome-dependent pathway. Once cellular DNA is damaged, MAPO1 is likely to be stabilized by escaping from the degradation pathway. This may constitute an obligatory step for inducing apoptosis in an MMR-dependent manner.

The database analysis revealed that MAPO1 is identical to FNIP2/FNIPL. This protein can bind to folliculin, encoded by the *FLCN* tumor suppressor gene, and also to AMP-activated protein kinase (AMPK) [16,17]. We have shown that folliculin and AMPK are involved in the $O^6$-methylguanine-induced apoptosis [24], and it has also been shown that MAPO1 is phosphorylated by AMPK *in vitro* [17]. These prompted us to investigate a possible role of folliculin and AMPK in the stabilization of MAPO1. When siRNA for the *FLCN* gene was introduced into HeLa MR cells, the amount of MAPO1 decreased considerably, even after treatment with MNU. In contrast, following the application of siRNA for the *AMPKα* gene, the MAPO1 level increased, and this increase was further enhanced after exposure to MNU. Even without MNU treatment, the administration of a kinase inhibitor of AMPK, compound C, increased the level of MAPO1. These results imply that MAPO1 may interact with folliculin and AMPK in a specific manner during the course of apoptosis. It was shown, indeed, that after treatment with MNU, folliculin retains its ability to stably bind with MAPO1, whereas AMPK dissociates gradually from MAPO1. In this way, folliculin and AMPK may stabilize MAPO1 in positive and negative manners,

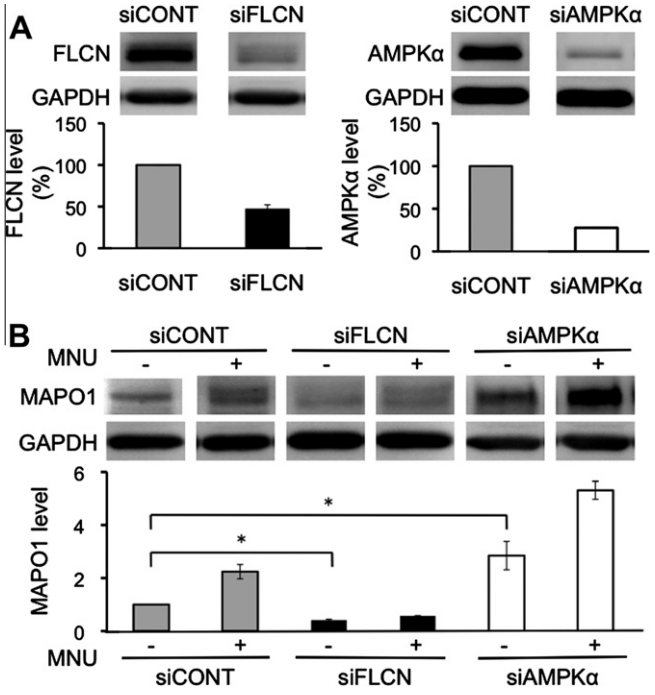


**Fig. 3.** The effects of *FLCN*- and *AMPK*α-knockdown on the level of MAPO1. (A) The expression levels of FLCN and AMPKα in cells treated with siRNAs. The whole cell extracts were prepared from HeLa MR cells transfected with control and siRNAs specific for the corresponding genes and applied to an immunoblotting analysis with antibodies for FLCN, AMPKα and GAPDH (loading control). The relative expression levels of FLCN and AMPKα in the cells treated with siRNAs are shown in the lower part. (B) The MAPO1 levels in the *FLCN*- and *AMPK*α-knockdown cells. Two days after transfection with siRNA, HeLa MR cells were treated with or without 1 mM MNU for 1 h and then incubated for three days. The cells were harvested and subjected to an immunoblotting analysis using antibodies that recognize MAPO1 and GAPDH. The relative expression levels of MAPO1 in the cells treated with siRNAs are shown in the lower part. $^{*}P < 0.01$.

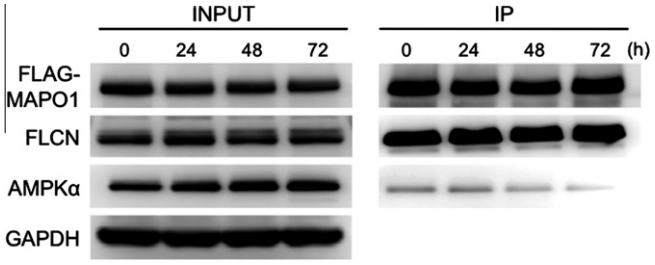


**Fig. 4.** A change in the interaction between MAPO1 and both FLCN and AMPKα after MNU treatment. HeLa MR cells expressing Flag-tagged MAPO1 were treated with 1 mM MNU for 1 h and withdrawn at 0, 24, 48 and 72 h of postincubation. Whole cell extracts (INPUT) were used for immunoprecipitation with anti-Flag M2 antibody beads (IP). The materials were subjected to SDS–PAGE, transferred to a membrane and immunoblotted using antibodies that recognize Flag-tag, FLCN, AMPKα and GAPDH.

respectively, during the process of apoptosis induction. Stabilization of the MAPO1 and FLCN complex may be achieved by the modification of these proteins, since it is known that MAPO1/FNIPL binds more tightly to the phosphorylated form of FLCN [17,27]. It is worthwhile to clarify the molecular processes in which MAPO1 is activated through the interaction with folliculin and AMPK in the signaling pathway of apoptosis.

## Acknowledgments

We thank Drs. R. Fujikane, Y. Takagi, D. Setoyama, H. Hayakawa, and R. Ito (Fukuoka Dental College, Japan) for helpful discussion. This work was supported by MEXT-Grants-in-Aid and MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2008–2012.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.11.064.

## References

[1] C. Coulondre, J.H. Miller, Genetic studies of the lac repressor. IV. Mutagenic specificity in the lacI gene of *Escherichia coli*, J. Mol. Biol. 117 (1977) 577–606.
[2] T. Ito, T. Nakamura, H. Maki, M. Sekiguchi, Roles of transcription and repair in alkylation mutagenesis, Mutat. Res. 314 (1994) 273–285.
[3] B. Demple, A. Jacobsson, M. Olsson, P. Robins, T. Lindahl, Repair of alkylated DNA in *Escherichia coli*. Physical properties of $O^6$-methylguanine-DNA methyltransferase, J. Biol. Chem. 257 (1982) 13776–13780.
[4] B.J. Glassner, G. Weeda, J.M. Allan, J.L. Broekhof, N.H. Carls, I. Donker, B.P. Engelward, R.J. Hampson, R. Hersmus, M.J. Hickman, R.B. Roth, H.B. Warren, M.M. Wu, J.H. Hoeijmakers, L.D. Samson, DNA repair methyltransferase (Mgmt) knockout mice are sensitive to the lethal effects of chemotherapeutic alkylating agents, Mutagenesis 14 (1999) 339–347.
[5] H. Kawate, K. Ihara, K. Kohda, K. Sakumi, M. Sekiguchi, Mouse methyltransferase for repair of $O^6$-methylguanine and $O^4$-methylthymine in DNA, Carcinogenesis 16 (1995) 1595–1602.
[6] K. Sakumi, A. Shiraishi, S. Shimizu, T. Tsuzuki, T. Ishikawa, M. Sekiguchi, Methylnitrosourea-induced tumorigenesis in MGMT gene knockout mice, Cancer Res. 57 (1997) 2415–2418.
[7] A. Shiraishi, K. Sakumi, M. Sekiguchi, Increased susceptibility to chemotherapeutic alkylating agents of mice deficient in DNA repair methyltransferase, Carcinogenesis 21 (2000) 1879–1883.
[8] T. Tsuzuki, K. Sakumi, A. Shiraishi, H. Kawate, H. Igarashi, T. Iwakuma, Y. Tominaga, S. Zhang, S. Shimizu, T. Ishikawa, et al., Targeted disruption of the DNA repair methyltransferase gene renders mice hypersensitive to alkylating agent, Carcinogenesis 17 (1996) 1215–1220.
[9] H. Kawate, R. Itoh, K. Sakumi, Y. Nakabeppu, T. Tsuzuki, F. Ide, T. Ishikawa, T. Noda, H. Nawata, M. Sekiguchi, A defect in a single allele of the Mlh1 gene causes dissociation of the killing and tumorigenic actions of an alkylating carcinogen in methyltransferase-deficient mice, Carcinogenesis 21 (2000) 301–305.
[10] P. Branch, G. Aquilina, M. Bignami, P. Karran, Defective mismatch binding and a mutator phenotype in cells tolerant to DNA damage, Nature 362 (1993) 652–654.
[11] M. Hidaka, Y. Takagi, T.Y. Takano, M. Sekiguchi, PCNA-MutSalpha-mediated binding of MutLalpha to replicative DNA with mismatched bases to induce apoptosis in human cells, Nucleic Acids Res. 33 (2005) 5703–5712.
[12] A. Kat, W.G. Thilly, W.H. Fang, M.J. Longley, G.M. Li, P. Modrich, An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair, Proc. Natl. Acad. Sci. USA 90 (1993) 6424–6428.
[13] K. Ochs, B. Kaina, Apoptosis induced by DNA damage O6-methylguanine is Bcl-2 and caspase-9/3 regulated and Fas/Caspase-8 independent, Cancer Res. 60 (2000) 5815–5824.
[14] Y. Takagi, M. Takahashi, M. Sanada, R. Ito, M. Yamaizumi, M. Sekiguchi, Roles of MGMT and MLH1 proteins in alkylation-induced apoptosis and mutagenesis, DNA Repair (Amst.) 2 (2003) 1135–1146.
[15] K. Komori, Y. Takagi, M. Sanada, T.H. Lim, Y. Nakatsu, T. Tsuzuki, M. Sekiguchi, M. Hidaka, A novel protein, MAPO1, that functions in apoptosis triggered by $O^6$-methylguanine mispair in DNA, Oncogene 28 (2009) 1142–1150.
[16] H. Hasumi, M. Baba, S.B. Hong, Y. Hasumi, Y. Huang, M. Yao, V.A. Valera, W.M. Linehan, L.S. Schmidt, Identification and characterization of a novel folliculin-interacting protein FNIP2, Gene 415 (2008) 60–67.
[17] Y. Takagi, T. Kobayashi, M. Shiono, L. Wang, X. Piao, G. Sun, D. Zhang, M. Abe, Y. Hagiwara, K. Takahashi, O. Hino, Interaction of folliculin (Birt–Hogg–Dube gene product) with a novel Fnip1-like (FnipL/Fnip2) protein, Oncogene 27 (2008) 5339–5347.
[18] M.L. Nickerson, M.B. Warren, J.R. Toro, V. Matrosova, G. Glenn, M.L. Turner, P. Duray, M. Merino, P. Choyke, C.P. Pavlovich, N. Sharma, M. Walther, D. Munroe, R. Hill, E. Maher, C. Greenberg, M.I. Lerman, W.M. Linehan, B. Zbar, L.S. Schmidt, Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt–Hogg–Dube syndrome, Cancer Cell 2 (2002) 157–164.
[19] C.D. Vocke, Y. Yang, C.P. Pavlovich, L.S. Schmidt, M.L. Nickerson, C.A. Torres-Cabala, M.J. Merino, M.M. Walther, B. Zbar, W.M. Linehan, High frequency of somatic frameshift BHD gene mutations in Birt–Hogg–Dube-associated renal tumors, J. Natl. Cancer Inst. 97 (2005) 931–935.
[20] T.R. Hartman, E. Nicolas, A. Klein-Szanto, T. Al-Saleem, T.P. Cash, M.C. Simon, E.P. Henske, The role of the Birt–Hogg–Dube protein in mTOR activation and renal tumorigenesis, Oncogene 28 (2009) 1594–1604.
[21] Y. Hasumi, M. Baba, R. Ajima, H. Hasumi, V.A. Valera, M.E. Klein, D.C. Haines, M.J. Merino, S.B. Hong, T.P. Yamaguchi, L.S. Schmidt, W.M. Linehan, Homozygous loss of BHD causes early embryonic lethality and kidney tumor

development with activation of mTORC1 and mTORC2, Proc. Natl. Acad. Sci. USA 106 (2009) 18722–18727.
[22] D. Carling, The AMP-activated protein kinase cascade – a unifying system for energy control, Trends Biochem. Sci. 29 (2004) 18–24.
[23] D.G. Hardie, The AMP-activated protein kinase pathway – new players upstream and downstream, J. Cell Sci. 117 (2004) 5479–5487.
[24] T.H. Lim, R. Fujikane, S. Sano, R. Sakagami, Y. Nakatsu, T. Tsuzuki, M. Sekiguchi, M. Hidaka, Activation of AMP-activated protein kinase by MAPO1 and FLCN induces apoptosis triggered by alkylated base mismatch in DNA, DNA Repair (Amst.) 11 (2012) 259–266.
[25] T.P. Cash, J.J. Gruber, T.R. Hartman, E.P. Henske, M.C. Simon, Loss of the Birt–Hogg–Dube tumor suppressor results in apoptotic resistance due to aberrant TGFbeta-mediated transcription, Oncogene 30 (2011) 2534–2546.
[26] M. Scheffner, B.A. Werness, J.M. Huibregtse, A.J. Levine, P.M. Howley, The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53, Cell 63 (1990) 1129–1136.
[27] L. Wang, T. Kobayashi, X. Piao, M. Shiono, Y. Takagi, R. Mineki, H. Taka, D. Zhang, M. Abe, G. Sun, Y. Hagiwara, K. Okimoto, I. Matsumoto, M. Kouchi, O. Hino, Serine 62 is a phosphorylation site in folliculin, the Birt–Hogg–Dube gene product, FEBS Lett. 584 (2010) 39–43.