Antiapoptotic Function of Apoptosis Inhibitor 2-MALT1 Fusion Protein Involved in t(11;18)(q21;q21) Mucosa-Associated Lymphoid Tissue Lymphoma

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ABSTRACT

The t(11;18)(q21;q21) is a characteristic chromosomal translocation in mucosa-associated lymphoid tissue (MALT) type lymphoma, and this translocation results in fusion transcript of apoptosis inhibitor 2 (API2), also known as c-IAP2, and MALT translocation gene 1 (MALT1). Although the API2-MALT1 fusion protein has been shown to enforce activation of nuclear factor-kB signaling, its precise role in the apoptotic signaling pathway remains to be established. To identify proteins that bind the API2-MALT1 protein, we used communoprecipitation and SDS-PAGE, followed by liquid chromatography-electrospray ionization tandem mass spectrometry. As a result, three important regulators of apoptosis, Smac, HtrA2, and TRAF2, and three other proteins were identified as potential API2-MALT1-binding proteins. Immunoprecipitation analyses verified that API2-MALT1 indeed binds to both exogenous and endogenous Smac proteins. It is especially noteworthy that stably transfected API2-MALT1 significantly suppressed both UV- and etoposide-induced apoptosis in HeLa cells, thus demonstrating for the first time that API2-MALT1 indeed possesses antiapoptotic function. Furthermore, API2-MALT1 significantly suppressed Smac-promoted apoptosis in UV-irradiated HeLa cells. Thus, our results provide direct experimental evidence that API2-MALT1 can confer resistance to apoptosis, at least in part, by neutralizing apoptosis promoted by Smac.

INTRODUCTION

Extranodal lymphomas arising from the mucosa-associated lymphoid tissue (MALT) represent a subtype of B-cell non-Hodgkin’s lymphoma with a distinct clinicopathological entity (1). Because of their supposed cell of origin, they are now recognized and defined as extranodal marginal zone lymphomas of MALT type in the revised European-American classification of lymphoid neoplasms (REAL) and the recently published WHO classification of malignant lymphomas (2, 5). According to the International Non-Hodgkin’s Lymphoma Classification Project, MALT lymphoma comprises 7.6% of all non-Hodgkin’s lymphomas and represents one of the most common non-Hodgkin’s lymphomas (4). The majority of MALT lymphomas occur in the stomach, but this lymphoma may affect most organs, including the ocular adnexa, lung, salivary glands, thyroid, skin, and intestine.

Data on the molecular genetic mechanisms underlying the pathogenesis of MALT lymphomas are only now beginning to emerge. Their recurrent abnormalities include trisomies of chromosomes 3, 7, 12, and 18, t(1;14)(p22;q32), and t(11;18)(q21;q21) (5–9). The BCL10 gene was isolated from the breakpoint region of the t(1;14) in MALT lymphomas and subsequently shown to be proapoptotic (10, 11). On the other hand, the t(11;18) translocation is reported to be one of the most frequent and specific chromosomal translocations in MALT lymphomas (12), and a novel gene, named MALT1 or MLT, was recently cloned by ourselves and others from the breakpoint of t(11;18). This aberration has been found to result in the fusion of two genes, apoptosis inhibitor 2 [API2 (also known as c-IAP2)] at 11q21 and the novel gene MALT1 at 18q21 (13–15), generating the API2-MALT1 fusion protein. More recently, a novel t(14;18) translocation involving the immunoglobulin gene at 14q32 and the MALT1 gene at 18q21 has been reported (16, 17), and the actual frequency of this translocation in MALT lymphoma is roughly 10% (18, 19).

API2 is a member of the inhibitor of apoptosis (IAP) protein family, which includes X-IAP, AIP1 (c-IAP1), API2 (c-IAP2), and ML-IAP, and has three baculovirus IAP repeat domains, one caspase recruitment domain, and one RING finger domain (20, 21). MALT1 is a novel protein that contains a death domain, two immunoglobulin-like domains, and a caspase-like domain (13–15, 22). It was demonstrated that MALT1 and BCL10 form a strong complex and that these proteins synergize in nuclear factor (NF)-kB activity (22, 23). Together with the findings for BCL10 and MALT1 knockout mice, these results suggest that both MALT1 and BCL10 link antigen receptor signaling to NF-kB activation (24–26). The caspase-like domain of MALT1 was demonstrated to be essential for NF-kB activity (22, 23), but functions of the death domain and immunoglobulin-like domains remain to be identified. It was also demonstrated that API2-MALT1 can induce NF-kB activation through its homodimerization mediated via the API2 portion of the fusion protein, whereas full-length API2, MALT, or their truncated forms cannot (22, 23), raising the possibility that the oncogenic role of API2-MALT1 may be mediated by deregulated NF-kB activity. Given that API2 is a member of the IAP family, it can be hypothesized that API2-MALT1 may exert an antiapoptotic effect as well. However, experimental evidence for such an antiapoptotic effect has yet to be presented.

In this study, we first tried to identify the binding proteins of API2-MALT1 to further delineate its apoptotic and oncogenic functions. To this end, we used electrospray ionization tandem mass spectrometry analysis of the communoprecipitates of transiently transfected API2-MALT1 in cultured cells and were able to identify Smac (27, 28) as consisting of several API2-MALT1-binding proteins. We also demonstrated for the first time that API2-MALT1 can significantly suppress both UV- and etoposide-induced apoptosis in HeLa cells and that this suppression may be mediated, at least in part, by neutralizing apoptosis promoted by Smac.

MATERIALS AND METHODS

Plasmids and Antibodies. The plasmids encoding FLAG-API2-MALT1 (pcDNA3-FLAG-API2-MALT1 and PCXN2-FLAG-API2-MALT1) have been described elsewhere (29), as has the plasmid for COOH-terminal myc-tagged Smac (pcDNA3-Smac-myc) (30). To generate the plasmids for pRKK5-FLAG-API2ΔC (amino acids 1–442) and pRKK5-FLAG-MALT1ΔN (amino acids 217–813), the corresponding fragments were PCR amplified using KOD Taq polymerase from their full-length cDNAs, digested with the appropriate enzymes, and then subcloned into pRKK5-FLAG-N plasmids. Proper construction of these plasmids was confirmed by DNA sequencing with the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Foster City, CA). The pEGFP-C plas-
mid was purchased from Clontech. Anti-FLAG M2 monoclonal antibody and polyclonal antibody against myc (A-14) were obtained from Santa Cruz Biotechnology. Anti-FLAG monoclonal antibody-coupled agarose beads were obtained from Sigma (St. Louis, MO).

**Purification of API2-MALT1-Binding Proteins.** 293T cells were maintained in Iscove’s medium supplemented with 10% FCS in a 5% CO2 incubator at 37°C. The pcDNA3 FLAG-tagged API2-MALT1 plasmid or the empty plasmid was transiently transfected into 293T cells using Effectene Reagent (Qiagen K.K., Tokyo, Japan) according to the manufacturer’s instructions. At 24 h after transfection, the cells were homogenized in lysis buffer [10 mM Tris (pH 8.0), 120 mM NaCl, 5 mM EDTA, and 0.5% Triton X-100] with Complete Protease Inhibitors (Roche Diagnostics, Tokyo, Japan). The homogenate was centrifuged at 10,000 × g for 15 min. The soluble fraction of the suspension was then incubated for 3 h, immunoprecipitated with anti-FLAG M2-agarose beads (Sigma), and washed five times in lysis buffer without protease inhibitors. The fractions eluted with 0.2 M glycine-HCl (pH 2.8) were neutralized, concentrated by freeze drying, and separated by 10% SDS-PAGE (15 × 15 cm). The bands detected by Coomassie Brilliant Blue staining were excised for in-gel digestion.

**High-Performance Liquid Chromatography Tandem Mass Spectrometry Analysis.** In-gel digestion was performed by trypsin digestion at 35°C overnight as described elsewhere (31). The digest was analyzed directly by means of nanoscale high-performance liquid chromatography coupled to a tandem mass spectrometer (Q-Tof; Micromass) equipped with a nanoelectrospray ionization source. The eluate was analyzed by tandem mass spectrometry (Q-Tof2) performed by Applied Bioscience (Tokushima, Japan). A database search of tandem mass spectra was performed using a Mascot Search Program (Matrix Science Ltd., London, United Kingdom).

**Immunoprecipitation and Western Blot Analysis.** 293T cells were transiently transfected using Effectene Reagent (Qiagen K.K.) or LipofectAMINE 2000 reagent (GIBCO-BRL, Tokyo, Japan) according to the manufacturer’s instructions. For immunoprecipitation, a total of 8 × 10^5 293T cells was placed on a 3.5-cm dish or a 6-well dish, washed the following day, and then transfected with a total of 0.5–1.0 μg of plasmid DNA. After 6 h of incubation, the medium was replaced with fresh complete medium. At 24 h after transfection, the cells were homogenized in lysis buffer [20 mM HEPES (pH 7.4), 120 mM NaCl, 5 mM EDTA, 0.5% Triton X-100, and 10% glycerol] with Complete Protease Inhibitors (Roche Diagnostics). Cellular debris was removed by centrifugation at 10,000 × g for 20 min, and the supernatant was incubated first with anti-FLAG monoclonal antibody at 4°C for 2 h and then with protein G-Sepharose at 4°C for 2 h. Immunoprecipitates were washed five times in lysis buffer without protease inhibitors. After this, immunoprecipitates were lysed with 1/10 sample buffer [62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 5% mercaptoethanol, and 0.05% bromphenol blue] and boiled for 3 min. It should be noted that the lysis buffer solubilizes mitochondrial membranes and thus releases Smac without the need for any apoptotic stimuli.

The samples were separated electrophoretically on SDS-PAGE and transferred to a polyvinylidene difluoride membrane, and the membrane was visualized with an enhanced chemiluminescence detection kit (Amersham-Biotechnology). Anti-FLAG monoclonal antibody-coupled agarose beads and anti-FLAG M2-agarose beads (Sigma), and washed five times in lysis buffer without protease inhibitors. The fractions eluted with 0.2 M glycine-HCl (pH 2.8) were neutralized, concentrated by freeze drying, and separated by 10% SDS-PAGE (15 × 15 cm). The bands detected by Coomassie Brilliant Blue staining were excised for in-gel digestion.

**RESULTS**

**Identification of API2-MALT1-Binding Proteins.** The t(11;18) translocation, most commonly encountered in MALT lymphoma, was found to result in the fusion of an IAP, API2, and a novel protein, MALT1 (13–15). Although API2-MALT1 was shown to activate NF-κB activity through its homodimerization, it remains to be established whether this fusion protein can indeed exert an antiapoptotic function, which may be relevant to the pathogenesis of MALT lymphoma. As a first step in addressing this fundamental issue, we used a proteomic approach to identify API2-MALT1-binding proteins.

293T cells were transiently transfected with either an expression plasmid for NH2-terminal FLAG-tagged API2-MALT1 or an empty plasmid. Cell lysates were prepared and incubated with anti-FLAG monoclonal antibody-coupled agarose beads. After extensive washing, associated proteins were eluted under acidic conditions from the beads and separated by SDS-PAGE. Five candidate protein bands were observed in a Coomassie Blue-stained gel (Fig. 1), but not in the control immunoprecipitate. These bands were excised from the gel and digested in gel with trypsin. The digested peptides were then analyzed directly by nanoscale high-performance liquid chromatography coupled to tandem mass spectrometry equipped with a nano-electrospray ionization source. A database search of tandem mass spectra was performed with a Mascot Search Program.

Table 1 summarizes the peptides detected by mass spectrometry and their assigned proteins. Several peptides of HtrA2 and Smac were detected in bands D and E, respectively. The detection of HtrA2 and Smac as API2-MALT1-binding proteins is not surprising, in view of previous reports that these two proteins were identified as X-IAP-binding proteins (27, 28, 30, 32). HtrA2 is a mitochondrial serine protease and is released from mitochondria to the cytoplasm during apoptosis (30, 33–35). It also interacts with and cleaves IAPs, relieving caspase inhibition and promoting apoptosis (36, 37). Smac is also

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normally a mitochondrial protein and, like HtrA2, is released to the cytoplasm concurrent with cytochrome c release during apoptosis (27, 28). It was found to interact with the baculovirus IAP repeat domain of IAPs. The binding of Smac to IAPs relieves the binding of IAPs to caspases, thus promoting caspase-mediated apoptosis. Several peptides of TRAF2 were detected in band 2. Because TRAF2 plays a crucial role in the tumor necrosis factor-stimulated apoptotic pathway, further study is warranted to examine the role of API2-MALT1 in this pathway. In addition to these regulators of apoptosis, three other proteins, Hsc70 (heat shock 70-kDa protein 8), GRP75 (stress-70 protein), and KIAA1892, were also detected in bands A and C. Identification of two members of the heat shock protein family of 70 kDa (HSP70) is intriguing because they not only function as molecular chaperones to facilitate protein folding and oligomerization, but also have been shown to exert antiapoptotic activity (38). KIAA1892 is a novel hypothetical protein whose function is currently unknown but is presumed to have WD-40 repeats. Given the fact that this novel protein has the potential to bind API2-MALT1, it will be interesting to further examine its function in the context of apoptosis as well as NF-κB signaling.

Unexpectedly, no peptides corresponding to BCL10 were detected that have been shown to form a complex with MALT1 through immunoglobulin-like domains (22, 23). To clarify this point, we performed Western blot analysis of API2-MALT1 immunoprecipitate samples (one-fourth of the proteins shown in Fig. 1) using BCL10 antibody. This analysis could not detect any apparent band corresponding to BCL10 (data not shown). One explanation for this non-detection would be that the amount of BCL10, if any, was undetectable in Coomassie Blue staining or below the detection limit of Western blot analysis. It is also conceivable that API2-MALT1 may have bypassed the normal BCL10 signaling pathway.

**Interaction between API2-MALT1 and Smac.** To verify the interaction between API2-MALT1 and Smac, expression plasmids for NH2-terminal FLAG-tagged API2-MALT1 (F-API2-MALT1), COOH-terminal-deleted API2 (F-API2ΔC), NH2-terminal-deleted MALT1 (F-MALT1ΔN), or an empty plasmid were transiently transfected together with an expression plasmid for Smac with a COOH-terminal myc tag (Smac-myc) into 293T cells. Transfected cell lysates were immunoprecipitated with anti-FLAG monoclonal antibody and then immunoblotted with anti-Myc monoclonal antibody. As shown in Fig. 2, the mature form of Smac-myc was coinmunoprecipitated with API2-MALT1 and API2ΔC. This indicates that API2-MALT1 indeed associates with exogenously expressed Smac in the cells.

We next sought to examine whether API2-MALT1 interacts with endogenous Smac in cells. Expression plasmids for F-API2-MALT1, F-API2ΔC, F-MALT1ΔN, or an empty plasmid were transiently transfected into 293T cells. Transfected cell lysates were immunoprecipitated with anti-FLAG monoclonal antibody and then immunoblotted with anti-Myc monoclonal antibody. As shown in Fig. 3, endogenous Smac was also immunoprecipitated with API2-MALT1, which strongly suggests that the interaction between API2-MALT1 and Smac could be physiologically significant. Interaction of API2-MALT1 with endogenous HtrA2 and TRAF2 was also confirmed by immunoprecipitation analysis (data not shown).

**Table 1** List of proteins identified as API2-MALT1-binding proteins

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Bands</th>
<th>Peptides detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSC70 (HSP73)</td>
<td>Band A</td>
<td>VEIHANDQGNR, NQVAMNPNTNTVFDAK, RFDDAVVQVQDMK, HWFMVNVNPGFK, SFYPEEVSVMVLTK, ILNEPTAAAYGLDVKK, VQQTVQQLFGR</td>
</tr>
<tr>
<td>GRP75 (75-kDa glucose-regulated protein, Stress-75 protein)</td>
<td>Band B</td>
<td>YAMTAEACSR, LDQQKEIALSK, DLAMADEQK, ILYNCDGTR, YGVVMMTLTSPSIIALELQKR, AGLRPVDILAGEMQVNAEADVYEAVER</td>
</tr>
<tr>
<td>TRAF2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precursor HtrA2</td>
<td>Band C</td>
<td>TLLATGDDPNPSLAIYR, AVPSPPPASP, SQYNFADYVEK, LSLGDTYEAATVTPVADIAATLRR, EPLPTFLGR, QGEPFVAMGSPFALQNTTSGIVSSAORPAR, VTAGISFAIPSDDR</td>
</tr>
<tr>
<td>KIAA1892</td>
<td>Band D</td>
<td></td>
</tr>
<tr>
<td>HtrA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smac</td>
<td>Band E</td>
<td>SEPHSLSSALMR, AYVYTLTSLYR, MNSSEEDEMVMOVIGAR, LETTTWMTAVGLSEMAAAEAYQTGDAGQSITAR, LQVEEVIHQSLR, LAEAAJIEELR, AEEQEAYLRED</td>
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Fig. 1. Detection of apoptosis inhibitor 2 (API2)-MALT1-binding proteins. Overexpressed FLAG-tagged API2-MALT1 in 293T cells was affinity-purified using anti-FLAG M2 monoclonal antibody-coupled agarose beads. Eluted fractions were subjected to SDS-PAGE and stained with Coomassie Blue. Coeluted proteins with affinity-purified API2-MALT1 are represented by A–E.
API2-MALT1 Confers Resistance to UV- and Etoposide-Induced Apoptosis. Because API2 is known to be an IAP (also known as c-IAP2), one can speculate that API2-MALT1 has a similar antiapoptotic function as well. Because no experimental evidence has been presented for such a function, however, we decided to test API2-MALT1 for this purpose. We chose a HeLa cell line in which several apoptotic stimuli including UV irradiation, chemotherapeutic agents, and tumor necrosis factor rapidly induce typical apoptotic changes characterized by chromatin condensation and DNA fragmentation. First, an API2-MALT1 expression plasmid was transiently transfected into HeLa cells, but the fusion protein was barely detectable by Western blot analysis of the whole cell lysates (data not shown). Therefore, we decided to establish stable transfectants expressing API2-MALT1. To this end, we transfected its linearized expression plasmid (PCXN2-FLAG-API2-MALT1) into HeLa cells, selected the cells with Geneticin, and finally cloned them by limiting dilution procedure. As a result, two independent clones expressing API2-MALT1, designated API2-MALT1-1 and API2-MALT1-2, were obtained and used for additional studies. As controls, two independent clones transfected with an empty plasmid only, designated Mock-1 and Mock-2, were also established.

It was noteworthy that when exposed to UV irradiation and treated with etoposide, both clones expressing API2-MALT1 (API2-MALT1-1 and API2-MALT1-2) showed significant attenuation, compared with that of the two control clones, in both UV- and etoposide-induced apoptosis as determined by the chromatin condensation (Figs. 4A and 5A). Thus, this provides for the first time direct evidence that API2-MALT1 can indeed confer resistance to apoptosis. We also measured DEVDase activity in these transfectants. Similarly, when exposed to UV irradiation or treated with etoposide, the two clones expressing API2-MALT1 showed significant attenuation of DEVDase activity (Figs. 4B and 5B), almost compatible with and further supporting the results of the apoptosis assay.

Because Smac has been shown to promote apoptosis in response to several stimuli such as UV irradiation that trigger the mitochondria-mediated apoptotic pathway, we examined the role of Smac in UV irradiation-induced apoptosis of the stable transfectants expressing API2-MALT1. To do so, we transiently transfected an expression plasmid for Smac-myc with a green fluorescent protein expression plasmid into the stable transfectants expressing API2-MALT1. To this end, we transfected its linearized expression plasmid (PCXN2-FLAG-API2-MALT1) into HeLa cells, but the fusion protein was barely detectable by Western blot analysis of the whole cell lysates (data not shown). Therefore, we decided to establish stable transfectants expressing API2-MALT1. To this end, we transfected its linearized expression plasmid (PCXN2-FLAG-API2-MALT1) into HeLa cells, selected the cells with Geneticin, and finally cloned them by limiting dilution procedure. As a result, two independent clones expressing API2-MALT1, designated API2-MALT1-1 and API2-MALT1-2, were obtained and used for additional studies. As controls, two independent clones transfected with an empty plasmid only, designated Mock-1 and Mock-2, were also established.

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Because Smac has been shown to promote apoptosis in response to several stimuli such as UV irradiation that trigger the mitochondria-mediated apoptotic pathway, we examined the role of Smac in UV irradiation-induced apoptosis of the stable transfectants expressing API2-MALT1. To do so, we transiently transfected an expression plasmid for Smac-myc with a green fluorescent protein expression plasmid into the stable transfectants and then induced apoptosis by UV irradiation. Approximately 47–49% of Smac-transfected cells without expression of API2-MALT1 showed signs of apoptosis, as determined by the condensed chromatin. In contrast, only about 21–22% of Smac-transfected cells with stable expression of API2-MALT1 showed signs of apoptosis (Fig. 6). Thus, API2-MALT1 can confer significant resistance to apoptosis promoted by Smac in UV-irradiated HeLa cells.
Anti-blotting using anti-FLAG monoclonal antibody or anti-Myc polyclonal antibody or /H11006 /H9262 transiently transfected with a COOH-terminal myc-tagged Smac plasmid (2
B, /Image 44x152 to 296x321/).

Three distinct chromosomal translocations have been implicated in the pathogenesis of MALT lymphoma. The first, t(1;14), results in the juxtaposition of the –
known as API2-MALT1 (13
15). The more recently discovered third
second, t(11;18), results in the synthesis of a novel fusion protein,
which BCL10 expression is thought to be deregulated (10, 11). The
BCL10 juxtaposition of the
apoptosis was assessed by the presence of condensed chromatin. B. DEVase activity (5 × 10⁶ cells/sample) was also measured. Data represent
mean ± SD of triplicate samples.

FIG. 5. Apoptosis inhibitor 2 (API2)-MALT1 confers resistance to apoptosis and suppresses DEVDase activity induced by etoposide treatment. HeLa cells stably transfected with a FLAG-tagged API2-MALT1 plasmid (API2-MALT1-1 and API2-MALT1-2) or an empty plasmid (Mock-1 and Mock-2) were treated with etoposide (100 µM) or DMSO for 12 h. A. apoptosis was assessed by the presence of condensed chromatin. B. DEVase activity (5 × 10⁶ cells/sample) was also measured. Data represent mean ± SD of triplicate samples.

DISCUSSION

Antiproapoptotic Function of API2-MALT1

In MALT cases with t(14;18), was incapable of inducing NF-κB activity (22, 23). Thus, it remains to be established whether NF-κB activation by API2-MALT1 is indeed relevant to the pathogenesis of MALT lymphoma. Furthermore, no experimental evidence has been produced related to the fundamental question of whether API2-MALT1 can confer resistance to apoptosis.

In the present study, we were able to identify API2-MALT1-binding proteins and to demonstrate for the first time that API2-MALT1 does exert an antian apoptotic effect against UV irradiation- and etoposide treatment-induced apoptosis in HeLa cells. This antian apoptotic effect may be mediated, at least in part, by action against the Smac-promoted apoptotic pathway. Because several apoptotic inhibitors have been shown to be up-regulated by NF-κB activation (39), our data do not entirely exclude the possibility that the antian apoptotic effect may be mediated, in part, by the secondary up-regulation of such apoptotic inhibitors. In a recent study, we examined whether API2-
MALT1 has an antian apoptotic effect on murine interleukin 3-dependent hematopoietic Ba/F3 cells (27). For this purpose, stable transfectants expressing API2-MALT1 were established, and apoptosis was induced by interleukin 3 deprivation or UV irradiation. These results indicated that API2-MALT1 does not show obvious resistance to apoptosis induced by interleukin 3 deprivation or UV irradiation in Ba/F3 cells. Because NF-κB activation is generally thought to contribute to an antian apoptotic effect, we analyzed the nuclear NF-κB activity of these two cell lines by using the NF-κB ELISA kit (Oxford Biomedical Research, Inc.). However, this analysis revealed no significant difference in the amount of nuclear NF-κB between the two cell lines (data not shown), suggesting that the difference in antian apoptotic effect by API2-MALT1 may not be due to the difference in the cell lines’ nuclear NF-κB activity. One reason for the difference between the results obtained for the HeLa and Ba/F3 cells would be that these two cell lines do not always use the same apoptotic signaling pathway.

We also examined the stability of ectopically expressed API2, MALT1, and API2-MALT1 by Western blot analysis of the cell lysates with or without treatment with MG132, a proteasome inhibitor (27). It was found that MALT1 is rapidly degraded via the ubiquitin-proteasome pathway, as is the case with API2. On the synthesis of fusion, API2-MALT1 was readily detectable even without MG132, suggesting that this fusion protein becomes stable against the ubiquitin-proteasome pathway (27). Thus, increased stability of API2-MALT1 would be expected to augment counteraction against or synergy with the functions of its binding partners including Smac, HtrA2, and TRAF2.

To the best of our knowledge, this is the first report demonstrating that API2-MALT1 acts as an antian apoptotic regulator, which may be mediated, at least in part, by neutralizing apoptosis promoted by Smac. It stands to reason that Smac would not be the only target for the antian apoptotic effect exerted by API2-MALT1, and indeed, we have identified TRAF2, HtrA2, KIAA1892, and HSP70s as other potential API2-MALT1-binding proteins. Given that Smac, TRAF2, and HtrA2 function as bona fide regulators of apoptosis, it is tempting to speculate that the KIAA1892 protein and two Hsp70s exert the same effect. Because recent studies of the BCL10 and MALT1 knockout mice provide evidence that both BCL10 and MALT1 participate in linking antigen receptor signaling and NF-κB activation (29–31) and exogenously expressed API2-MALT1 was shown to enforce activation of NF-κB activity (27, 28), it will be of particular importance to pursue the possible roles of API2-MALT1-binding proteins in antigen receptor signaling. It is hoped that additional studies will provide further insight into the biological functions of API2-MALT1 and ultimately lead to new diagnostic and therapeutic advances for dealing with MALT lymphoma, a human lymphoma that has been attracting special attention.

FIG. 6. Apoptosis inhibitor 2 (API2)-MALT1 confers resistance to apoptosis by action against the Smac-mediated apoptotic pathway. HeLa cells stably transfected with a FLAG-tagged API2-MALT1 plasmid (0.5 µg) or an empty plasmid (0.5 µg) were transiently transfected with a COOH-terminal myc-tagged Smac plasmid (2 µg) and a pEGFP plasmid (0.5 µg), incubated for 24 h, and then stimulated with UV irradiation (200 J/m²). A. after 6 h, apoptosis was assessed by the presence of condensed chromatin and expressed as a percentage of apoptotic cells in total transfected green cells. Data represent mean ± SD from triplicate samples. B. whole cell lysates were analyzed with immuno-blotting using anti-FLAG monoclonal antibody or anti-Myc polyclonal antibody or anti-β-actin monoclonal antibody. Lane 1, wild-type HeLa cells; Lanes 2 and 3, HeLa cells with empty vector; Lanes 4 and 5, HeLa cells with API2-MALT1 stable expression.
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