行政院國家科學委員會專題研究計畫 成果報告

抗人類大腸直腸癌之分子訊息作用機制

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Morusin 抗人類大腸直腸癌之分子訊息作用機(2/2)

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計畫主持人：李政昌

本成果報告包括以下應繳交之附件：

□ 赴國外出差或研習心得報告一份
□ 赴大陸地区出差或研習心得報告一份
□ 出席國際學術會議心得報告及發表之論文各一份
□ 國際合作研究計畫國外研究報告書一份

執行單位：國立成功大學醫學院外科部

中華民國95年10月25日
Morusin 抗人類大腸直腸癌之分子訊息作用機 (2/2)

Antitumor molecular signaling mechanism of Morusin in Human colorectal cancer cells

計畫編號：NSC 94-2314-B-006-016
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主持人：李政昌　國立成功大學醫學院外科部

一、中文摘要

Morusin 是由 M. australis (Moraceae) 的根部中分離出的成份，桑白皮的粗抽出物在台灣民間常被用於改善原住民的癌症，我們以前的研究已證明對人類大腸直腸細胞 (HT-29及HCT116)、人類肝癌細胞 (Hep 3B) 及人類乳癌細胞 (MCF-7)之生長具有抑制的作用。抑制大腸直腸癌細胞生長主要是使細胞膜內側的磷脂胺酸有翻轉至外側細胞膜，細胞的sub-G1期的DNA有增加，DNA片段化現象出現，並造成細胞核濃縮及出現凋亡小體現象使細胞凋亡，並活化caspase-8、caspase-9、caspase-3。本年度的研究發現Morusin 可使HT-29的caspase-8活化tBid由細胞質轉至粒線體而降低Bcl-XL表現並使粒線體膜電位喪失，而造成cytochrome c及Smac/DIABLO由粒線體中釋放到細胞質中。另外，Morusin抑制IKK-α、IKK-β及IkB-α之磷酸化作用、而增加IkB-α的表現並抑制NF-κB轉入核內及抑制核內DNA的結合能力。抑制Ku70的調節作用及抑制NF-κB在核內的調節作用而致影響XIAP之表現而引起細胞死亡之信息，簡言之，我們的研究顯示Morusin引起大腸直腸細胞HT-29的細胞凋亡可能經由caspases和NF-κB亡途徑。

關鍵詞: Morusin, 人類大腸直腸癌, 細胞凋亡, caspases, 粒線體, cytochrome c, Smac/DIABLO, Bcl-2, XIAP, PKCs, Akt/PI3K, NF-κB

Abstract

Morusin, isolated from root bark of Morus australis, has been used as a traditional folk remedy for treatment of cancers in Taiwan. Our previous studies demonstrated that morusin significantly inhibited the growth of human colorectal cancer cells (HT-29 and HCT 116), human hepatocellular carcinoma cells (Hep 3B), and human breast adenocarcinoma cells (MCF-7). The susceptibility of cells to morusin was caused by apoptosis through phosphatidylserine exposure, increase of DNA fragmentation, nuclear morphologic change, and sub-G1 content. The molecular mechanism of morusin on apoptosis was associated with the activation of caspase-8, caspase-9 and caspase-3. The present study demonstrates that activation of caspase-8 leads to the translocation of tBid from cytosol to mitochondria, and significant decrease in anti-apoptotic Bcl-XL in mitochondrial. The loss of mitochondrial membrane potential results in the release of cytochrome c and Smac/DIABLO from mitochondria to cytosol in HT-29 cells. Moreover, morusin
inhibits the phosphorylation of IKK-α, IKK-β and IκB-α, increases expression of IκB-α, and suppresses nuclear translocation of NF-κB and its DNA binding activity. Downregulation of Ku70 and NF-κB-mediated suppression of XIAP further insures the signs of cell death. In summary, our present results indicate that morusin induces cell death in human colorectal cancer HT-29 cells via caspases and NF-κB pathway.

**Keywords**: Morusin, human colorectal cancer, apoptosis, caspases, mitochondria, cytochrome c, Smac/DIABLO, Bcl-2, XIAP, PKCs, Akt/PI3K, NF-κB

二、緣由與目的：

Human colorectal cancer is one of the most common malignant tumors which causes major public health problems in western countries (1) and in Taiwan. Development of colon cancer is a multiple process (2) and the activation of oncogene K-ras is an early event (3-5). A drug called 5-fluorouracil is the most widely used medicine in chemotherapy of human colon cancer (6, 7). However, the therapeutic response of this medicine is limited at 15% in advanced human colon cancer. Therefore, treatment concentrates on chemoprevention in at-risk and chemotherapy for the inhibition of human colorectal cancer is very meaningful. An important aim in development of cancer therapeutic agents is to lower their side effects, thus led us to search natural products from Chinese traditional herbs for potential anticancer agents. Flavonoids, naturally plant products, have referred as mild tender drugs. They are phenolic plant pigments and are considered to have many pharmacological functions including anti-inflammatory, antioxidants and antitumor activity (8, 9). Among these actions, the antitumor effect may be mediated by the different cell cycle arrest (10) and the induction of apoptosis in tumor cells (11). Morusin, a natural product, isolated from the root bark of Morus australis (Moraceae) that has been used as a traditional Taiwanese folk remedy for treatment human cancer. Morusin is a prenylated flavonoids (chemical entities have an isoprenyl) containing a flavonoid backbone structure (12). Previous report had shown that morusin significantly inhibits arachidonic acid-, PAF-, and collagen-induced platelet aggregation (13). Notably, no study on the antitumor activity of morusin has been reported. Therefore, we will focus on exploring the antitumor activity and molecular mechanism of morusin in human colorectal cancer.

Apoptosis, programmed cell death, is a regulated process involving activation of molecular episode and induction of cell death characterized by morphological changes, DNA fragmentation, phosphatidylserine externalization, and generation of apoptotic bodies (14-19). The apoptotic body is engulfed by phagocyte and leads little or no inflammation (20). Apoptotic cell death occurs at an execution phase characterized by condensation and fragmentation of nuclear chromatin with internucleosomal cleavage of DNA (21). The induction of apoptosis is a potentially promising approach for cancer therapy (22). Apoptosis can be induced by stimuli including radiation, hyperthermia, and chemotherapeutic agents including etoposide, adriamycin, mitoxanthrone, cisplatin, and 5-fluorouracil (5-FU) (14, 16, 23-26). Apoptosis is carried out through the activation of intracellular cysteine protease known as caspase (27, 28). Caspases are expressed as precursors activated in a cascade following a pro-apoptotic stimulus (28). Notably,
Lassus et al. reported that caspase-2 is required for chemotherapeutic agents-induced apoptosis in human cancer cell lines (29, 30), and the activation of this apical caspase-2 is required for translocation of Bax to the mitochondria and for the releases of cytochrome c and Smac/DIABLO (second mitochondria-derived activator of caspases/direct IAP binding protein with low pl) (29, 30). Caspase-9 activation requires binding of the precursor to a complex of two proteins, Apaf-1 and cytochrome c, and is dependent on the hydrolysis of dATP or ATP (31-33). Activated Caspase-9 can further activate the executioner caspases including caspase-3 and caspase-7 that promote apoptosis by cleaving cellular substrates (34-36). The substrates of caspase-3 include poly (ADP-ribose) polymerase (PARP) (37, 38), DNA-dependent protein kinase (39, 40) and DFF45 (41). Recent studies have also indicated that apoptotic DNA fragmentation and associated nuclear changes are largely attributable to a Mr 40,000 nuclease termed CAD3 also known as CPAN or DFF40 (41-43), which functions as a nuclease and degrades nucleosomal DNA (28). In nonapoptotic cells, CAD/CPAN/DFF40 remains inactive because it is bound to its natural inhibitor ICAD (43). ICAD can be expressed as two isoforms, a Mr 45,000 isoform (ICAD-L/DFF45) and a Mr 35,000 isoform (ICAD-S/DFF35), caused by alternative splicing (41, 44). Both ICAD-L/DFF45 and ICAD-S/DFF35 can bind and inhibit CAD/CPAN/DFF40 (22, 41, 42).

The inhibitor of apoptosis IAP gene family encoded proteins appear as a key intrinsic inhibitors of the caspase cascade, and represent decisive regulatory factors in apoptosis signaling (34, 45, 46). X-linked inhibitor of apoptosis (XIAP; also known as MIHA, ILP) inhibits mature caspase-9 and caspase-3, respectively (47-49). The caspase-inhibiting effects of XIAP are antagonized by another apoptosis promoting mitochondrial protein, called Smac/DIABLO that synthesized in the cytoplasm and is targeted to the intermembrane space of mitochondria (50, 51). Smac/DIABLO competes with the small subunit of caspase-9 for XIAP, and tears XIAP away to relieve their inhibitory effect on both initiator and effector caspases, while cytochrome c stimulates downstream caspase cascades (50-55).

The Bcl-2 family of proteins is a critical regulator of apoptosis that can either inhibit (Bcl-2 and Bcl-XL) or promot (Bax and Bad) apoptosis (56-58). The mitochondria-mediated apoptosis is initiated by the release of Smac/DIABLO and cytochrome c into the cytosol and this process is control by Bcl-2 family members (28 review ref). The location of Bcl-2 on the outer membrane of mitochondria raises the possibility that its function may be related to the function of mitochondria, which have been implicated in apoptosis (59-63). Previous studies accumulating evidences demonstrated that the mitochondria plays an essential role in many forms of apoptosis (64) by releasing apoptotic factors, such as cytochrome c (65, 66), apoptosis-inducing factor (AIF) (67), Smac/DIABLO (50, 51) from the intermembrane space of mitochondria into the cytosol, which activate the downstream executional phase of apoptosis. Mitochondrial membrane depolarization is an early event of apoptosis, and the overexpression of antiapoptotic Bcl-2 family prevents this event (62) (63, 68). Both Bcl-2 and Bcl-XL block all of the changes in mitochondria including mitochondrial membrane potential loss, and the
releases of Smac/DIABLO and cytochrome c (62, 69-71). Recombinant Bax is reported to induce cytochrome c release and mitochondrial membrane potential losses (69, 70). This effect can be inhibited by cyclosporin A (a permeability transition pore inhibitor) that targets mitochondrial cyclophilin D (72). The cyclosporin A-sensitive permeability transition (PT) pore has been implicated in Bax/Bak-mediated cytochrome c releases and mitochondrial membrane potential losses (64).

Nuclear factor-κB (NF-κB), a mammalian transcription factor, is pivotal in regulation of many genes involved in immune system, inflammatory responses, tumour development, as well as in control of cell proliferation and apoptosis (73-78). An inactive NF-κB resides in the cytoplasm bound to an inhibitory protein known as IκB (73). Activation of NF-κB is triggered by extracellular stimuli, and then IκBα is phosphorylated and proteolytically processed by proteasomes and other proteases (79). This proteolytic process allows NF-κB to translocate from cytosol to the nucleus, and therefore bind to the promoter region of the target gene (80). Recent work provides direct evidence that NF-κB mediates a critical antiapoptotic signal and leads to rescue cells from apoptosis, and therefore contribute to oncogenesis (75, 81). Other lately evidence showed that the activation of NF-κB is through Ras and phosphatidylinositol-3-kinase (PI3-K) involving activation of Akt and IKK (82). Phosphorylation and degradation of IκBα, and the activation of NF-κB result in the translocation of NF-κB from cytosol to the nucleus. These processes induce NF-κB DNA-binding activity(83, 84). Moreover, current reports indicate clearly that NF-κB-dependent transcriptional activation of cIAP1 and cIAP2 (inhibitor of apoptosis protein) play a pivotal role in regulating apoptosis, and directly inhibit the activity of caspase 3, 7 and 9 (45, 85, 86). There are several evidences indicating that PKC and Akt kinase are crucial factors for cell death or survival in vivo (87, 88) and in vitro (89-91), and these two kinases are able to phosphorylate Bad protein (92-94). The phospho-Bad can interact with 14-3-3 protein and restrict in inhibition of mitochondrial located Bcl-2 family and inhibit its function (72, 95, 96).

Our preliminary study demonstrated that the concentration of 50% inhibition (IC50) of morusin on human colorectal cancer HT-29 cell growth was 4.76 µM (Table 1). The inhibiting efficacy of colony formation on HT-29 cell was evaluated at 14 days post-treatment of PF-C at various concentrations. As shown in Table 1, the IC50 value for colony formation was 1.9 µM. Moreover, susceptibility of the cells to morusin may be caused by apoptosis, which was demonstrated by increase of Sub-G1 content and DNA fragmentation. Therefore, the specific goal of the present study is to obtain new insights into the antitumor activity and signaling pathways of morusin on human colorectal cancer HT-29 cells in vitro and in vivo. We will continue our preliminary data, morusin-induced apoptosis in human colorectal cancer HT-29 cells will be investigated to conform again. Hypodiploid DNA will be determined by flow cytometry and agarose gel electrophoresis by ladder pattern of DNA fragments. The phosphatidylserine export to the outer leaflet of plasma membrane of morusin-treated HT-29 cells will also be investigated by flow cytometry. In addition, morusin induced phenotypic changes in HT-29 cell nucleus will be
stained with Hoechst 33258 examining by fluorescence microscopy. To test if morusin-induced apoptosis depends on protein synthesis or gene expression, cells treated with cyclohexamide or cyclosporin A prior addition of morusin will be examined. Furthermore, the cell cycle distribution in morusin treated-HT-29 cells will be analyzed by flow cytometry. The molecular mechanism of morusin induced programmed cell death will be elucidated. In order to investigate if apoptosis is through AIF, cytochrome c, Smac/DIABLO and caspase pathway, AIF, cytochrome c and Smac/DIABLO releases and caspase (such as caspase-2, -8, -9 and -3) activity will be examined by using Western blotting and imaged by laser scan confocal microscopy. To examine the effect of morusin on apoptotic signal pathway, the following apoptotic proteins such as, Bid, Bcl-2, Bcl-XL, Bad, Bak, cleavage PARP and DFF45 will be determined. Since, multiple mechanisms of action are reported to involve in the regulation of cell death for almost all anticancer agents, to determine if the observed antitumor effects involving the suppression of NF-κB activity, PKC and XIAP expression become important. The total, cytosol, cell membrane and nuclear fractions of proteins will be extracted. We will also examine if PKC, Akt/PI3K and NF-κB could underlie the cytotoxic effects of morusin in HT-29 cells. To investigate whether PI3K/Akt inhibition is required for morusin-induced apoptosis in HT-29 cells, we will perform Western blot analysis for PI3K and phospho-Akt expression. To test the possibility that NF-κB will involve in morusin-induced apoptosis in HT-29 cells, we will determine the nuclear extracts from morusin-treated HT-29 cells. The NF-κB nuclear translocation will be examined by Western blot, EMSA and confocal microscopy image analysis. Moreover, we will also investigate upstream signaling of NF-κB pathway including NIK, IKK and IκB protein expression. Finally, the therapy of morusin on colorectal cancer cell implanted severe combined immunodeficiency (SCID) mice will be examined. These results of this study will help us to unveil the effects of morusin on human colorectal cancer cells and provide an alternative therapy for this disease.

三、結果與討論：

Morusin changes ∆ψm and affects mitochondria-related protein expression in HT-29 cells. Apoptotic death signal can proceed via caspases and mitochondria, and the polarization of mitochondrial membrane can be regulated by the members of Bcl-2 family (27, 64, 97). To examine whether the Bcl-2 family proteins participate in morusin-induced apoptosis of human colorectal cancer, subcellular fractions of HT-29 cells were subjected to immunoblotting. As shown in Fig. 1, the expression of pro-apoptotic Bid decreased in the cytosol while its activated form of truncated Bid (tBid) increased in the mitochondria. Translocation of another pro-apoptotic protein, Bax, from cytosol to mitochondria was also revealed at 12-72 h (Fig. 1). Significant decrease in anti-apoptotic Bcl-XL was displayed in mitochondria at 48 h and reached the lowest level (60% decrease) at 72 h (Fig. 1). However, total expression of Bcl-XL was not affected (Fig. 1). Ku70 is another crucial factor for apoptosis (98). Downregulation of Ku70 has been reported to enhance Bax-mediated apoptosis, whereas overexpression of Ku70 inhibits the process (99, 100). In Fig. 2, decrease in the level of total and cytosolic Ku70 was exhibited at 48 h, and the expression of the cytosolic Ku70 was almost undetected at 72 h. However, the nuclear expression of Ku70 remained the same (Fig. 2). To ascertain the role of mitochondria in
morusin-treated HT-29 cells, \( \Delta \psi_m \) was evaluated by staining with rhodamine 123 and then analyzed by flow cytometry. As shown in Fig. 3, time-related increase in the percentage of cells with damaged mitochondria was observed, ranging from 2.4% at time zero to 77.4% at 72 h after treatment of 28.6 \( \mu \)M morusin. Change of \( \Delta \psi_m \) has been reported to increase the permeability of outer mitochondrial membrane which allows efflux of apoptogenic proteins to the cytosol (97). To address this, the release of cytochrome c and Smac/DIABLO from mitochondria to cytosol was determined by immunoblotting. As shown in Fig. 4, increase of cytochrome c and Smac/DIABLO in cytosol was first observed at 12 h and reached the highest level at 72 h. As expected, the expression of cytosolic Smac/DIABLO has been shown to bind XIAP and neutralizes its anti-apoptotic activity (4). Cytosolic Smac/DIABLO was determined by immunoblotting.

As shown in Fig. 4, increase of cytochrome c and Smac/DIABLO in cytosol was first observed at 12 h and reached the highest level at 72 h. As expected, the expression of mitochondrial cytochrome c and Smac/DIABLO decreased with time (Fig. 4). Cytosolic Smac/DIABLO has been reported to bind XIAP and neutralizes its anti-apoptotic activity (101). In this study, decrease in total XIAP was first detected at 24 h and reached the lowest at 72 h in morusin-treated HT-29 cells (Fig. 4).

Apoptosis can be regulated by members of caspase in which caspase-8 and -9 are initiator caspases and caspase-3 is classified into an effector caspase (27, 102). As shown in Fig. 5, morusin significantly increased the cleavage form of activated caspase-8 at 12 h of treatment, and this activation status sustained to 72 h. Increase in the activation form of cleaved caspase-9 and caspase-3 was also observed at 12-72 h (Fig. 5).

Morusin decreases NF-\( \kappa \)B activity in HT-29 cells. NF-\( \kappa \)B is a transcription factor, and has been shown to promote cell survival by upregulation of XIAP expression (103, 104). In the present study, significantly decrease in total XIAP protein expression was displayed, which implies the involvement of NF-\( \kappa \)B in morusin-induced apoptosis. To ascertain the role NF-\( \kappa \)B, nuclear translocation of NF-\( \kappa \)B in HT-29 cells was determined by western blotting. As shown in Fig. 6, morusin dramatically decreased the nuclear NF-\( \kappa \)B p65 subunit at 3 h of treatment and reached the lowest level at 6-24 h. Consistently, the significantly decrease in DNA-binding activity of nuclear NF-\( \kappa \)B was also detected by EMSA at 3 h (Fig. 7). The NF-\( \kappa \)B band was confirmed by observing the elimination of NF-\( \kappa \)B band in the presence of unlabeled \( \kappa \)B (Fig. 7, lane 8). Unlabeled mutant \( \kappa \)B had no effect (Fig. 7, lane 7). The involvement of NF-\( \kappa \)B subunits were also confirmed by showing the suppression of NF-\( \kappa \)B bands in the presence of anti-p50 and/or anti-p65 antibodies (Fig. 7, lane 9-11). To characterize the signaling pathway in the inhibition of NF-\( \kappa \)B activity in morusin-treated HT-29 cells, change of its upstream regulators were determined. As shown in Fig. 8, decrease in IkB-\( \alpha \) phosphorylation and increase in total IkB-\( \alpha \) protein expression was observed. Furthermore, morusin significantly decreased the phosphorylation and total protein expression of both IKK-\( \alpha \) and IKK-\( \beta \) (Fig. 8). These results suggest that downregulation of NF-\( \kappa \)B activity is involved in human colorectal carcinoma HT-29 cells through the dephosphorylation of IKK-\( \alpha \), IKK-\( \beta \) and IkB-\( \alpha \), and augment of IkB-\( \alpha \) and reduction of IKK-\( \alpha \) and IKK-\( \beta \) protein expression.

The role of NF-\( \kappa \)B in drug resistance has been reported (105). NF-\( \kappa \)B activity is elevated in colorectal cancer cells (106, 107). Administration of NF-\( \kappa \)B inhibitor enhances the tumoricidal response of the cells (105, 108). 5-fluorouracil (5-FU) is the most common and effective chemotherapeutic agents for the treatment of colorectal cancer (109). However, colon tumors are inherently resistant or develop resistance during treatment with 5-FU (110). Recently, a major mechanism for inherent and inducible chemoresistance has been suggested to associate with activation of NF-\( \kappa \)B (111, 112). In the present study, morusin not only induces apoptosis but significantly suppresses NF-\( \kappa \)B activity in HT-29 cells. The results suggests that given morusin in conjunction with 5-FU may potentiate the effect of 5-FU in patients with colorectal cancer.

In conclusion, morusin suppresses the
growth of human colorectal cancer HT-29 cells. Inhibition of constitutive NF-κB activity and induction of caspase-mediated apoptosis is exhibited. Morusin decreases the activity of p-IKK-α/p-IKK-β, p-IκB-α and then NF-κB in the cells. Activated caspase-8 causes the increase in mitochondrial tBid and Bax for the release of cytochrome c and Smac/DIABLO, and thus for the activation of caspase-9 and caspase-3 to promote the process of programmed cell death.

四、文献

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Table 1

The IC\textsubscript{50} effect of morusin on cell growth and colony formation on human tumor cells and human PBMCs

<table>
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<tr>
<th>Cell lines</th>
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<th>Clonogenic cell inhibition ((\mu\text{M/ml}))</th>
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<td>HT-29</td>
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<td>HCT 116</td>
<td>7.2 ± 0.8</td>
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<td>Hep 3B</td>
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<tr>
<td>HEK293</td>
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<tr>
<td>PBMC</td>
<td>29.8±0.1</td>
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\textsuperscript{a} Control cells were treated with DMEM. Experiments were repeated three times and each concentration of samples was conducted in eight replicates.

\textsuperscript{b} The experimental data are expressed as mean ± SEM.
Fig. 1

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<th>24</th>
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<td></td>
</tr>
<tr>
<td>Time (h)</td>
<td>C</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>48</td>
<td>72</td>
</tr>
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<td>---------</td>
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<td>Cell number</td>
<td></td>
<td></td>
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<tr>
<td>Morusin (28.6 µM)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C</td>
<td>2.4 ± 0.7%</td>
<td>18.6 ± 1.5%</td>
<td>22.6 ± 2.4%</td>
<td>36.0 ± 4.2%</td>
<td>66.2 ± 4.3%</td>
<td>77.4 ± 6.6%</td>
</tr>
<tr>
<td>24 h</td>
<td>4.1 ± 1.1%</td>
<td>12.8 ± 2.4%</td>
<td>21.4 ± 3.7%</td>
<td>39.9 ± 3.6%</td>
<td>67.1 ± 7.5%</td>
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</table>

Fluorescence intensity (µM)
**Fig. 4**

<table>
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<tr>
<th>Time (h)</th>
<th>C 12 24 48 72</th>
<th>kDa</th>
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<tbody>
<tr>
<td><strong>Cytosolic cytochrome c</strong></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td><strong>Mitochondrial cytochrome c</strong></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td><strong>Cytosolic Smac/DIABLO</strong></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td><strong>Mitochondrial Smac/DIABLO</strong></td>
<td></td>
<td>25</td>
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<tr>
<td><strong>Total XIAP</strong></td>
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<td>57</td>
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Morusin (28.6 μM)
Fig. 5

<table>
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<th>12</th>
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<th>72</th>
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<tbody>
<tr>
<td>Caspase-8</td>
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<tr>
<td>Cleaved caspase-8</td>
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<tr>
<td>RACK1</td>
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<td>Caspase-9</td>
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<td>Cleaved caspase-9</td>
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<tr>
<td>Caspase-3</td>
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<td>Cleaved caspase-3</td>
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Morusin (28.6 μM)
Fig. 6

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<th>Time (h)</th>
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<th>Nuclear Ku70</th>
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<tr>
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<td>0.12</td>
<td>0.04</td>
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<tr>
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Morusin (28.6 µM)
Fig. 7

<table>
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<tr>
<th>Probe alone</th>
<th>Morusin (28.6 µM)</th>
<th>Time(h)</th>
<th>Mutant</th>
<th>α p50</th>
<th>α p65</th>
<th>α p50+p65</th>
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<td></td>
<td></td>
<td>0 3 6 12 24</td>
<td>κB</td>
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<table>
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<tr>
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<tr>
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<tr>
<td>5</td>
<td>0.1</td>
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<tr>
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<td>0.9</td>
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NF-κB

Free probe

18
Fig. 8

Morusin (28.6 µM)

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<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-IκB-α</td>
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kDa

0 12 3 6

37

85

87