Degradation of Survivin by the X-linked Inhibitor of Apoptosis (XIAP)-XAF1 Complex*

Received for publication, January 26, 2007, and in revised form, June 22, 2007 Published, JBC Papers in Press, July 5, 2007, DOI 10.1074/jbc.M700776200

Vinay Arora1, Herman H. Cheung1,2, Stéphanie Plenchette3, O. Cristina Micali, Peter Liston, and Robert G. Korneluk4
From the Apoptosis Research Centre, Children’s Hospital of Eastern Ontario Research Institute, Ottawa, Ontario K1H 8L1, Canada

X-linked inhibitor of apoptosis (XIAP)-associated factor 1 (XAF1) is a putative tumor suppressor in which expression is significantly reduced in human cancer cell lines and primary tumors. The proapoptotic effects of XAF1 have been attributed to both caspase-dependent and -independent means. In particular, XAF1 reverses the anti-caspase activity of XIAP, a physiological inhibitor of apoptosis. We further investigated the function of XAF1 by examining its relationship with other IAPs. Immunoprecipitation studies indicate that XAF1 binds to XIAP, cIAP1, cIAP2, Livin, TsIAP, and NAIP but not Survivin, an IAP that prevents mitotic catastrophe and in which antiapoptotic activity is exerted through direct XIAP interaction and stabilization. We found that overexpressed XAF1 down-regulates the protein expression of Survivin. Under these conditions, Survivin expression was restored in the presence of the proteasome inhibitor MG132 or a XIAP RING mutant that is defective for ubiquitination. In addition, RNA interference (RNAi) in ubiquitin-protein isopeptide ligase (E3) activity, suggesting some inhibitor of apoptosis (IAP) that prevents mitotic catastrophe and in which antiapoptotic functions by binding to and inhibiting caspases. In contrast, the physical interaction between Survivin and caspases appears to be mostly physiologically irrelevant. Instead, the mechanism of Survivin in promoting cytotoxicity may lie in its ability to associate with XIAP and enhance its stability by antagonizing function of the RING E3 ligase domain (3).

XIAP-associated factor 1 (XAF1) was identified as a nuclear protein that binds to XIAP and suppresses its anti-caspase activity (4, 5), thereby reversing the protective effect of XIAP overexpression in cancer cell lines. The expression of XAF1 is significantly reduced in cancer cell lines (4, 6) and in several primary malignancies (7, 8) as a result of promoter hypermethylation (9, 10). Moreover, reactivation of xaf1 by DNA methylation inhibitors sensitizes cancer cells to apoptosis-inducing agents (11, 12). Conditionally replicative adenovirus encoding XAF1 was found to have potent anti-tumor efficacy in an animal model of colorectal carcinoma (13).

Interferons (IFN) are the most broadly active cytokines that are used for cancer treatment (14). Interestingly, xaf1 is an IFN-stimulated gene that sensitizes TRAIL-induced apoptosis in several tumor cell lines (15). Moreover, Survivin expression is down-regulated after interferon-β1a treatment in T-lymphocytes from multiple sclerosis patients (16). The potential link between this induction of the IAP antagonist XAF1 and the regulation of Survivin has not been determined.

In this report, we investigated the relationship between XAF1 and different members of the IAP family. We found that XAF1 interacts with XIAP, cIAP1, cIAP2, Livin, TsIAP, and NAIP but not Survivin. In particular, the formation of the XIAP-XAF1 complex mediates Survivin down-regulation through a complex containing XIAP, supporting dual roles for XAF1 in apoptosis and mitotic catastrophe.

The inhibitor of apoptosis (IAP) family members XIAP and Survivin have emerged as promising cancer therapeutic targets (1, 2). Survivin is a structurally and functionally unique IAP in that it contains a single baculoviral IAP repeat and no RING domain and has evolved dual roles in mitosis and apoptosis. XIAP is a potent endogenous inhibitor of apoptosis that functions by binding to and inhibiting caspases. In contrast, the physical interaction between Survivin and caspases appears to be mostly physiologically irrelevant. Instead, the mechanism of Survivin in promoting cytotoxicity may lie in its ability to associate with XIAP and enhance its stability by antagonizing function of the RING E3 ligase domain (3).

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Harvesting**—All media were supplemented with heat-inactivated 10% fetal bovine serum (Invitrogen), 1 unit/ml penicillin, and 1 μg/ml streptomycin (Fisher), and all cell cultures were maintained at 37 °C in 5% CO2 in 100-mm tissue culture grade dishes (Corning) and passaged every 3–4 days. X-A3, X-linked IAP; NAIP, neuronal apoptosis-inhibitory protein; E3, ubiquitin-protein isopeptide ligase; RNAi, RNA interference; GFP, green fluorescent protein; siRNA, small interfering RNA; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; XAF1, XIAP-associated factor 1;
48–72 h. 293T human embryonic kidney (Invitrogen), A-375 melanoma (ATCC), and SF-539 glioblastoma (Brain Tumor Research Center, University of California, San Francisco) were maintained in Dulbecco’s minimal essential medium (Invitrogen), with SF-539 supplemented with 1% nonessential amino acids and 1% glutamine. SK-MEL-5 melanoma (ATCC) and ACHN renal carcinoma (ATCC) cells were maintained in minimum essential medium (Invitrogen) supplemented with 1% nonessential amino acids (Invitrogen). SK-N-BE(2) neuroblastoma cells were maintained in Dulbecco’s minimal essential medium/F-12 Ham’s (1:1) (Invitrogen) supplemented with 1% nonessential amino acids and 1% glutamine. Human ovarian cancer cells OVCAR3 and OV2008 were maintained in RPMI 1640 media (Invitrogen) with OVCAR3 supplemented with 10 mM HEPES, 20% fetal bovine serum, 1% sodium pyruvate, and 0.01 mg/ml bovine insulin.

Cells were collected by centrifugation and lysed in 50 mM Tris-HCl, pH 8.0, containing 150 mM NaCl, 1% Triton X-100, 1 mM NaF, and a mixture of protease inhibitors consisting of 0.1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, 5 µg/ml pepstatin A, and 10 µg/ml leupeptin. The cell lysates were incubated on ice for 20 min followed by centrifugation at 12,000 × g for 15 min to pellet the insoluble cell debris. Alternatively, to obtain whole cell homogenates, cells were harvested and lysed in 1% SDS, 62.5 mM Tris-HCl, pH 6.8, containing protease inhibitors. The cell lysates were incubated at room temperature for 15 min, sonicated in a water bath for 10 min, and subsequently boiled for 5 min. Protein content was determined by the Bio-Rad protein assay using bovine serum albumin as a standard.

Plasmid DNA Constructs and Transient Transfections—pcDNA3–6myc-XIAP, cIAP1, and cIAP2 were described previously (17). Full-length coding sequence of NAIP, TsiAP, and Survivin were subcloned into pcDNA3 or pcDNA3–6myc using the BamHI and XhoI ligation sites. The primers 5'-CGGA-TCATGGGACCTAAAGACAGT-3' and 5'-AGGAGATCTGCGCAGGCAAAACGCACCAC-3' were used to amplify the coding region of XIAP. XIAP mutant 6myc-pcDNA3-XIAPH467A in which the replacement of His467 residue with Ala results in the loss of XIAP E3 ubiquitin ligase activity (18) was generated by using the Site-Directed Mutagenesis system (Invitrogen).

Plasmid DNA Constructs and Transient Transfections—pcDNA3–6myc-XIAP, cIAP1, and cIAP2 were described previously (17). Full-length coding sequence of NAIP, TsiAP, and Survivin were subcloned into pcDNA3 or pcDNA3–6myc using the BamHI and XhoI ligation sites. The primers 5'-CGGA-TCATGGGACCTAAAGACAGT-3' and 5'-AGGAGATCTGCGCAGGCAAAACGCACCAC-3' were used to amplify the coding region of XIAP. XIAP mutant 6myc-pcDNA3-XIAPH467A in which the replacement of His467 residue with Ala results in the loss of XIAP E3 ubiquitin ligase activity (18) was generated by using the Site-Directed Mutagenesis system (Invitrogen). xaf1 shRNA Plasmid Construction and Stable Transfection—

**XAF1 Down-regulates Survivin**

The design and construction of the shRNA clones against xaf1 was performed according to a method described previously (19). In brief, U6 RNA PolIII promoter (D. McManus, University of Ottawa) was PCR-amplified using a forward primer (5'-AGGAGATCTGCGCAGGCAAAACGCACCAC-3') and a variety of 96-nucleotide-long shRNA XAF1 primers as described previously (20) prior to cloning into a TOPO-TA vector (Invitrogen). The shRNA was subcloned into a pcDNA3 vector devoid of its cytomegalovirus promoter and transected into SF-539 cells as described above. Stable clones were selected in Dulbecco’s minimal essential medium supplemented with 600 µg/ml G418 (Invitrogen).

**Recombinant Adenoviral Vectors and Cell Infection**—The adenoviral vectors adeno-lacZ and adeno-xaf1 were generated as reported previously (4). GFP-RNAi-luciferase and GFP-RNAi-XIAP were gifts from Dr. Dan McManus (University of Ottawa). The design and construction of the RNAi clones against XIAP has been previously described (20). Cells were seeded at low density in 60–100-mm dishes and transduced at a multiplicity of infection of 50 plaque-forming units/cell. Transduced cells were collected 48 h post-infection or exposed to further treatment with various agents before harvesting.

**Immunoprecipitation**—Cells were collected by centrifugation and resuspended in lysis buffer (50 mM Tris-HCl, pH 6.8, 150 mM NaCl, 1% Triton X-100) containing protease inhibitors. Lysates were incubated on ice for 20 min and then centrifuged at 12,000 × g for 20 min. Immunoprecipitation was performed using the anti-XIAP antibodies or the anti-c-Myc immunoprecipitation kit (Sigma) as instructed by the manufacturer’s protocol. Preimmunized nonspecific rabbit serum was used as a control.

**Western Immunoblotting**—For immunoblotting, equal amounts of SDS-solubilized samples were separated by SDS-PAGE and transferred to nitrocellulose as described previously (21). Following protein transfer, individual proteins were detected by Western immunoblotting using the following antibodies with the stated dilutions recognizing: XAF1 at 1:500, XIAP at 1:1000, Survivin (Cell Signaling) at 1:1000, Myc tag (StressGen) at 1:1000, and β-Actin (Sigma) at 1:5000. β-Actin immunoreactivity was used as a loading control. Mouse monoclonal anti-XAF1 antibody was generated against full-length GST-XAF1 following standard techniques and recognized a reactive band at ~34 kDa. Polyclonal anti-XIAP antibodies were generated by immunizing rabbits with GST-XIAP fusion protein in RIBI adjuvant (Sigma) as described previously (4). Bound primary antibodies were reacted with secondary antibodies conjugated with Alexa Fluor® 680 (Molecular Probes) or with IRDye™ 800 (Rockland) at 1:10,000, and the infrared fluorescence signals were detected and analyzed using the Odyssey® infrared imaging system (LI-COR).

**Caspase Activity Assay**—Cells were incubated in lysis buffer without protease inhibitors for 30 min at 4 °C and centrifuged (10,000 × g, 15 min, 4 °C). The resulting supernatant containing the solubilized proteins was quantified and stored at ~80 °C. Caspase 3-like activity was measured in a 96-well microplate by incubating equal amount of proteins (20 or 50 µg) in the reaction buffer (100 mM HEPES, 1 mM EDTA, 0.1%...
CHAPS, 10% glycerol, 20 mM dithiothreitol) in the presence of 100 H9262 M fluorogenic peptide substrate DEVD-AMC (7-amino-4-methylcoumarin) (BioMol). Fluorescence released from the substrate was monitored each minute at 37 °C for 60 min in a spectrofluorimeter microplate reader (POLARstar Galaxy, Fisher Scientific). Enzymatic activities were expressed as initial velocities (fluorescence intensity/min/mg) and normalized to basal controls.

IFN-γ Treatment—Cells were treated with vehicle or 500 units/ml recombinant IFN-γ (Sigma) for 24 h and harvested for subsequent analysis.

Proteasome Inhibitor Treatment—Transfected 293T cells were treated with 10 μM proteasomal inhibitor MG-132 (Calbiochem) at 20 h posttransfection for 4 h. The cells were harvested at 24 h posttransfection.

RESULTS

XAF1 Interacts with RING-bearing IAPs and NAIP but Not Survivin—XAF1 has been shown to bind XIAP and subsequently antagonize the anti-caspase activity of XIAP (4). To extend this observation, we analyzed for XAF1 interactions with other members of the IAP family, specifically cIAP1, cIAP2, Livin, TsIAP, Survivin, and NAIP. After 48 h of cotransfection with expression constructs that encode for XAF1 and Myc-tagged IAPs, proteins were extracted from 293T cells and subjected to immunoprecipitation with anti-c-Myc-agarose suspension (20 μl) or nonspecific rabbit serum as a control (lane 11) was performed and subsequently analyzed by Western immunoblotting (IB) for XAF1. Cells transfected with pCI-XAF1 alone were used as a control. XAF1 was shown to bind to all IAPs tested except Survivin (lane 8). Results are representative of at least three separate experiments.

An XIAP-XAF1 Complex Mediates Survivin down-regulation—A complex of XIAP and Survivin is known to promote increased XIAP stability against ubiquitination-mediated proteasomal destruction and synergistic inhibition of apoptosis (3). These previous results suggest that if XAF1 were to affect Survivin, it would likely be indirectly through its interaction with XIAP. Therefore, we next determined whether XAF1 might...
The E3 ligase of XIAP is essential for Survivin down-regulation by XAF1. A, 293T cells were transfected with combinations of pcDNA3-Survivin, pcDNA3–6myc-XIAP, and pCI-XAF1. Transfected cells were treated with 10 μM proteasomal inhibitor MG-132 for 4 h and harvested at 24 h for analysis by Western immunoblotting. MG-132 treated cells showed greater Survivin expression levels compared with untreated cells. B, protein expression levels of Survivin from A were quantified and plotted as average ± S.E. Number of samples is indicated inside the parentheses. C, 293T cells were transfected with combinations of pcDNA3-Survivin, pCI-XAF1, and pcDNA3–6myc-XIAP or the mutant construct pcDNA3–6myc-XIAP_H467A that expresses a XIAP lacking E3 ligase activity. The E3 inactive H467A mutation on XIAP rescued XAF1-mediated down-regulation of Survivin. D, protein expression levels of Survivin from C were quantified and plotted as average ± S.E. Number of samples is indicated inside the parentheses.

Figure 3. The E3 ligase of XIAP is essential for Survivin down-regulation by XAF1.

The E3 Ligase of XIAP Is Essential for Survivin Down-regulation by XAF1—XIAP contains a RING domain possessing E3 ligase activity that participates in the ubiquitin-proteasome pathway (18). We sought to determine whether XAF1-mediated down-regulation of Survivin might engage the ubiquitin-proteasome pathway. In the presence of the proteasome inhibitor MG132, XAF1 failed to induce Survivin degradation (Fig. 3, lane 3 versus 4). These results indicate that XAF1 regulates the level of Survivin by modulating XIAP function.

The E3 Ligase of XIAP is essential for Survivin down-regulation by XAF1. As shown in Fig. 4 (lane 1 versus 3), the up-regulation of XAF1 appears to promote a reduction of XIAP protein concomitant with Survivin degradation. This is consistent with a previous report demonstrating that Survivin is able to stabilize XIAP (3), and conversely, the loss of Survivin could lead to down-regulation of XIAP. We therefore investigated the potential contribution that this XIAP reduction has on etoposide-induced caspase activation by comparing XIAP with its E3 ligase mutant in the presence of XAF1 and Survivin. We found that irrespective of the E3 ligase status of XIAP, XAF1 was effective in reversing the inhibition of caspase activity (Fig. 5), either in the absence (lanes 4 and 5 versus 6 and 7) or in the presence of Survivin (lanes 9 and 10 versus 11 and 12). These data suggest that Survivin does not play a direct role in caspase-3 suppression. Moreover, forced XAF1 expression, either in complex with wild-type XIAP or E3 ligase mutant XIAP, results in caspase-3 activation.

IFN-mediated Survivin Down-regulation Requires XAF1 Induction—xafl is an IFN-stimulated gene in which expression can be induced in certain cell lines (15). To determine whether the induction of endogenous XAF1 by IFN-β down-regulates Survivin, we treated A-375, SK-MEL-5, ACHN, OVCAR3, and OV2008 with IFN-β for 24 h. We found that in response to IFN-β, XAF1 protein expression was elevated in A-375, SK-MEL-5, and ACHN but not in OVCAR3 and OV2008 (Fig. 6, A and B). Consistent with a role for XAF1 in regulating Survivin levels, IFN-β treatment in A-375 and SK-MEL-5 cells, and in ACHN at a lesser extent, the induced XAF1
XAF1 Down-regulates Survivin

expression correlated with Survivin down-regulation. Conversely, the lack of XAF1 induction in OVCAR3 and OV2008 correlated with unchanged levels of Survivin protein (Fig. 6, A and B). To investigate the endogenous formation of a complex that contains XIAP, XAF1, and Survivin, we have immunoprecipitated the XIAP-containing complex in the presence or absence of IFN-β. We found that XAF1 and Survivin were parts of the complex only after IFN-β treatment (Fig. 6C).

To confirm a role for XAF1 in regulating Survivin levels in response to IFN-β, we generated xaf1 knock-out stable glioblastoma SF-539 clones that are nonresponsive to IFN-β-mediated XIAP induction (22). In accord with the above results, XAF1 induction led to down-regulation of Survivin in SF-539; the silencing of this induction by RNA interference in turn prevented the down-regulation (Fig. 6, D and E). Together, these results demonstrate that XAF1 is important for the down-regulation of Survivin in response to IFN-β in physiological condition.

DISCUSSION

XAF1 is a candidate tumor suppressor that antagonizes XIAP, a critical suppressor of apoptosis (5). Herein we have established that the endogenous XAF1-XIAP complex promotes association of Survivin with XIAP, thereby reversing the stabilizing effects of Survivin on XIAP. As a result, the RING E3 ligase of XIAP is activated, and the subsequent proteasomal degradation of Survivin ensues. Moreover, we show that the induction of XAF1 is a prerequisite for the down-regulation of Survivin in response to IFN-β treatment.

We found that XAF1 interacts with all IAPs tested with the exception of Survivin. XIAP is a bona fide physiological represor of both initiator and effector caspases (1), and the binding of XAF1 to XIAP reverses the anti-caspase activity of XIAP (4). Potentially, by interacting with target IAPs, XAF1 can antagonize their cytoprotective functions. The antiapoptotic activity of cIAP1 and cIAP2 is attributed to the neutralization of Smac (23) and their anti-caspase activity is considered inconsequential (24). This raises the possibility that the IAP-antagonist property of XAF1 might involve the reversal of Smac neutralization, perhaps by displacing Smac. Alternatively, in a manner more analogous to XAF1-mediated Survivin down-regulation, XAF1 could potentially promote IAP-mediated Smac degradation by the ubiquitin-proteasome pathway (25, 26).

The formation of an XIAP-Survivin complex appears to depend on the presence of XAF1. In contrast to a recent report (3), we were unable to see a direct interaction between XIAP and Survivin in the absence of XAF1. The previous report used GST fusion proteins to assess the direct binding between XIAP and Survivin. However, this could be problematic as another recent report has shown that GST tags could confer baculoviral IAP repeat domains with artifactual properties because of the propensity of the GST tag to oligomerize, thereby leading to the formation of higher order structures that could lead to unnatural interactions (24). Furthermore, the possibility of other proteins being involved in XIAP-Survivin interaction in vivo cannot be excluded, especially because it was shown by an earlier study that XIAP-Survivin interaction in vivo was enhanced by
an apoptotic signal (3). In our study, it is quite possible that the expression of XAF1 may itself serve as the necessary apoptotic trigger that stimulates XIAP-Survivin interaction.

We found that XAF1 activates the latent E3 activity of the XIAP RING domain and triggers the destruction of Survivin. These results are in accord with Survivin protein expression being regulated, in part, by the ubiquitin-proteasome pathway (27). We demonstrate that XAF1 complexes with XIAP, thereby reversing the inhibition of ubiquitination of XIAP by Survivin and activating the XIAP E3 ligase to target and promote Survivin degradation. Survivin is part of the chromosomal passenger complex that is essential for accurate spindle check point function, mitotic chromosomal movements, and proper execution of cytokinesis (28). Survivin

\[ \text{Survivin}^\text{−/−} \]

mouse embryos dis-
XAF1 Down-regulates Survivin

play an increase in ploidy, cytokinesis failure, and large microtubule bundles (29), and RNAi studies show that the loss of Survivin results in mitotic catastrophe (30, 31). Because XAF1 has dual roles in suppressing XIAP and degrading Survivin, it would be interesting to determine to what extent XAF1 induction would recapitulate the cellular phenotype that arises from the loss of Survivin.

XAF1 is an antagonist of XIAP anti-caspase activity (4). The fact that the induction of XAF1 down-regulates Survivin, which in turn could destabilize XIAP (3), raises the prospect that XAF1 could also indirectly regulate XIAP levels because of the loss of Survivin. However, we found that the inhibition of etoposide-induced caspase activity by XIAP and E3 ligase inactive XIAP was reversed similarly by XAF1 (Fig. 5), either in the presence or absence of Survivin. This suggests that the direct antagonism of XIAP by XAF1 is important to the suppression of caspase-3 activation and can be independent of Survivin.

The expression ratio of XIAP and XAF1 could control cell fate. In neonates, a relative high level of XAF1 expression compared with XIAP correlates with sensitivity of motoneurons to axotomy-induced neuronal cell death (32). Because destabilization of XIAP due to Survivin reduction by XAF1 would alter expression ratio between XIAP and XAF1, it is possible that in certain cellular contexts, the indirect regulation of XAF1 on XIAP level might play a larger role in determining cellular outcome.

Although it is clear that XAF1 is required for the degradation of Survivin by XIAP, it remains possible that additional factors might be involved. For instance, XAF1 might instead bind to an intermediate that modulates the E3 ligase activity of XIAP. In such a scenario, the binding of XAF1 to the intermediate, but not XIAP, would be the prerequisite for Survivin down-regulation. This issue can be addressed by identifying and characterizing XAF1 mutants that are incapable of binding to XIAP, or alternatively, by finding and employing molecules that could disrupt XAF1–XIAP interaction.

We found that the induction of XAF1 is essential for Survivin degradation in response to IFN-β. IFN-dependent induction of XAF1 has been demonstrated previously to influence strongly cellular sensitivity to TRAIL-induced apoptosis (15). The down-regulation of Survivin in breast cancer cells by peroxisome proliferator-activated receptor-γ (33), in glioma and neuroblastoma cells by troglitazone (34), and in hepatoma cells by RNAi (35) sensitizes these cells to TRAIL-induced apoptosis. Moreover, xaf1 knock-out stable glioblastoma SF-539 cells had completely lost their IFN-β-mediated TRAIL sensitivity (22). Our findings suggest that IFN-mediated sensitization to TRAIL-induced apoptosis is, at least in part, because of the induction of XAF1 that then promotes degradation of Survivin by XIAP.

Both XIAP and Survivin are promising targets for anti-cancer therapy (1, 2, 36). Notably, antisense oligodeoxynucleotides AEG-35156 and ISIS-23722, which target XIAP and Survivin, respectively, are currently undergoing clinical trials (1, 36). The ability of XAF1 in promoting degradation of Survivin and its potential antagonism against other IAPs, as well as its inhibitory effects on XIAP anti-caspase activities, all support the concept that XAF1 could also be an attractive target for therapeutic development.

Acknowledgments—We thank the staff and students of the Apoptosis Research Centre. We also thank Lynn Kelly for the production of adenoviruses and Dr. Dan McManus for reagents.

REFERENCES

1. Cheung, H. H., LaCasse, E. C., and Korneluk, R. G. (2006) Clin. Cancer Res. 12, 3238–3242
2. Altieri, D. C. (2003) Nat. Rev. Cancer 3, 46–54
3. Dohi, T., Okada, K., Xia, F., Willford, C. E., Samuel, T., Welsh, K., Marusawa, H., Zou, H., Armstrong, R., Matsuzawa, S., Salvesen, G. S., Reed, J. C., and Altieri, D. C. (2004) J. Biol. Chem. 279, 34087–34090
4. Liston, P., Fong, W. G., Kelly, N. L., Toji, S., Miyazaki, T., Conte, D., Tamai, K., Craig, C. G., Mburney, M. W., and Korneluk, R. G. (2001) Nat. Cell Biol. 3, 128–133
5. Plenchette, S., Cheung, H. H., Fong, W. G., LaCasse, E. C., and Korneluk, R. G. (2007) Curr. Opin. Investig. Drugs 8, 469–476
6. Fong, W. G., Liston, P., Rajcan-Separovic, E., St Jean, M., Craig, C., and Korneluk, R. G. (2000) Genomics 70, 113–122
7. Ng, K. C., Campos, E. I., Martinka, M., and Li, G. (2004) J. Invest. Dermatol. 123, 1127–1134
8. Ma, T. L., Ni, P. H., Zhong, J., Tan, J. H., Qiao, M. M., and Jiang, S. H. (2005) Chin. J. Dig. Dis. 6, 10–14
9. Byun, D. S., Cho, K., Ryu, B. K., Lee, M. G., Kang, M. J., Kim, H. R., and Chi, S. G. (2003) Cancer Res. 63, 7068–7075
10. Lee, M. G., Huh, J. S., Chung, S. K., Lee, J. H., Byun, D. S., Ryu, B. K., Kang, M. J., Chae, K. S., Lee, S. J., Lee, C. H., Kim, J. I., Chang, S. G., and Chi, S. G. (2006) Oncogene 25, 5807–5822
11. Fang, X., Liu, Z., Fan, Y., Zheng, C., Nilsson, S., Egevad, L., Ekmek, P., and Xu, D. (2006) Int. J. Cancer 118, 2485–2489
12. Reu, F. J., Bae, S. I., Cherkassky, L., Leeman, D. W., Lindner, D., Beaulieu, N., MacLeod, A. R., and Borden, E. C. (2006) J. Clin. Oncol. 24, 3771–3779
13. Qi, R., Gu, J., Zhang, Z., Yang, K., Li, B., Fan, J., Wang, H., He, Z., Qiao, L., Lin, Z., and Liu, X. Y. (2007) Cancer Gene Ther. 14, 82–90
14. Borden, E. C. (2005) J. Interferon Cytokine Res. 25, 511–527
15. Leaman, D. W., Chawla-Sarkar, M., Vyas, K., Reheman, M., Tamai, K., Toji, S., and Borden, E. C. (2002) J. Biol. Chem. 277, 28504–28511
16. Sharief, M. K., and Semra, Y. K. (2002) Arch. Neurol. 59, 1115–1121
17. Liston, P., Roy, N., Tamai, K., Lefebvre, C., Baird, S., Cherton-Horvat, G., Farahani, R., McLean, M., Ikeda, J. E., Mackenzie, A., and Korneluk, R. G. (1996) Nature 379, 349–353
18. Yang, Y., Fang, S., Jensen, J. P., Weissman, A. M., and Ashwell, J. D. (2000) Science 288, 874–877
19. Paddison, P. J., Caudy, A. A., Bernstein, E., Hannon, G. J., and Conklin, D. S. (2002) Genes Dev. 16, 948–958
20. McManus, D. C., Lefebvre, C. A., Cherton-Horvat, G., St-Jean, M., Kandimalla, E. R., Agrawal, S., Morris, S. J., Durkin, J. P., and LaCasse, E. C. (2004) Oncogene 23, 8105–8117
21. Cheung, H. H., and Gurd, J. W. (2001) J. Neurochem. 78, 524–534
22. Miculi, O. C., Cheung, H. H., Plenchette, S., Hurley, S. L., Liston, P., LaCasse, E. C., and Korneluk, R. G. (2007) BMC Cancer 7, 52
23. Wilkinson, J. C., Wilkinson, A. S., Scott, F. L., Csomos, R. A., Salvesen, G. S., and Duckett, C. S. (2004) J. Biol. Chem. 279, 51082–51090
24. Eckelman, B. P., and Salvesen, G. S. (2006) J. Biol. Chem. 281, 3254–3260
25. Ma, L., Huang, Y., Song, Z., Feng, S., Tian, X., Wu, D., Qiu, X., Heese, K., and Wu, M. (2006) Cell Death Differ. 13, 2079–2088
26. Hu, S., and Yang, X. (2003) J. Biol. Chem. 278, 10055–10060
27. Zhao, J., Tenev, T., Martins, L. M., Downward, J., and Lemoine, N. R. (2000) J. Cell Sci. 113, 4363–4371
28. Vagnarelli, P., and Earnshaw, W. C. (2004) Chromosoma (Berl.) 113, 211–222
29. Wheatley, S. P., and McNeish, I. A. (2005) Int. Rev. Cytol. 247, 35–88
30. Carvalho, A., Carmena, M., Sambade, C., Earnshaw, W. C., and Wheatley, S. P. (2003) J. Cell Sci. 116, 2987–2998
31. Lens, S. M., Woltuis, R. M., Klompmaker, R., Kauw, J., Agami, R., Brummelkamp, T., Kops, G., and Medema, R. H. (2003) *EMBO J.* **22**, 2934–2947
32. Perrelet, D., Perrin, F. E., Liston, P., Korneluk, R. G., MacKenzie, A., Ferrer-Alcon, M., and Kato, A. C. (2004) *J. Neurosci.* **24**, 3777–3785
33. Lu, M., Kwan, T., Yu, C., Chen, F., Freedman, B., Schafer, J. M., Lee, E. J., Jameson, J. L., Jordan, V. C., and Cryns, V. L. (2005) *J. Biol. Chem.* **280**, 6742–6751
34. Schultze, K., Bock, B., Eckert, A., Oevermann, L., Ramacher, D., Wiestler, O., and Roth, W. (2006) *Apoptosis* **11**, 1503–1512
35. Nakao, K., Hamasaki, K., Ichikawa, T., Arima, K., Eguchi, K., and Ishii, N. (2006) *Oncol. Rep.* **16**, 389–392
36. Reed, I. C. (2006) *Nat. Clin. Pract. Oncol.* **3**, 388–398