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Parkin induces apoptotic cell death in TNF-α-treated cervical cancer cells

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Many malignant tumors become resistant to tumor necrosis factor-alpha (TNF-α)-induced cell death during carcinogenesis. In the present study, we examined whether parkin acts as a tumor suppressor in HeLa cells, a human cervical cancer cell line resistant to TNF-α-induced cell death. TNF-α-treatment alone did not affect HeLa cell viability. However, expression of parkin restored TNF-α-induced apoptosis in HeLa cells. Increased cell death was due to the activation of the apoptotic pathway. Expression of parkin in TNF-α-treated HeLa cells stimulated cleavage of the pro-apoptotic proteins caspase-8, -9, -3, -7 and poly ADP ribose polymerase (PARP). In addition, parkin expression resulted in decreased expression of the caspase inhibitory protein, survivin. These results suggest that parkin acts as a tumor suppressor in human cervical cancer cells by modulating survivin expression and caspase activity. We propose that this pathway is a novel molecular mechanism by which parkin functions as a tumor suppressor. [BMB Reports 2012; 45(9): 526-531]

INTRODUCTION

Parkin protein (parkin) was originally implicated in Parkinson's disease (1). The parkin gene (*PARK2*) is located on chromosome 6q25.2-6q27 and the loss of heterozygosity (LOH) in 6q26 is found in ovarian, breast, hepatocellular and squamous cell lung cancers (2-4). Since these reports, the association between cancer and genetic mutations in parkin has become an active area of research. In a variety of cancers including brain, liver, colorectal, ovarian, cervical, pancreas, kidney and breast cancers, alternative transcripts were found due to gene deletion and duplication in *PARK2* (5-10). In acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) and some colorectal cancer cells, abnormal methylation in *PARK2* gene results in a decreased expression of parkin (7, 11). Recent reports show that decreases in parkin expression have an essential role in tumorigenesis suggesting that parkin is a putative tumor suppressor (5-7, 9-12). Consistent with this notion, overexpression of parkin in hepatocarcinoma, glioblastoma, lung cancer, breast cancer and colon cancer cell lines repressed cell growth (7-10, 12). These studies attracted interest in studying the mechanisms by which parkin acts as a tumor suppressor. In breast cancer, parkin stabilizes microtubules and increases susceptibility to anti-cancer agents (13). In the breast cancer cell line MCF7, parkin reduces cell growth by inducing expression of cyclin-dependent kinase 6 (CDK6) (8). In glioblastoma and other cancers, parkin overexpression results in the degradation of ubiquitin-mediated cyclin E and subsequent cell cycle arrest (9). Simultaneous mutation in both *PARK2* and *APC* genes dramatically accelerates colorectal carcinogenesis (7). Parkin seems to exert different effects in different types of cancers and therefore the mechanisms by which parkin suppresses tumorigenesis vary and many questions remain unanswered.

Immune cells secrete various cytokines in response to cancers (14). As the cancer develops, they become resistant to anti-tumor cytokines such as tumor necrosis factor-alpha (TNF-α) (15). TNF-α is a pro-inflammatory cytokine secreted by macrophages and TNF-α exerts a suppressive effect on tumors (16). However, tumors can become TNF-α resistant and eventually most malignant tumors become TNF-α resistant (17, 18). For these reasons, many malignant tumors can escape from TNF-α-induced cell death (17). These observations highlight TNF-α resistance as a key factor in tumor progression.

Cervical cancer is one of the most common cancers among women worldwide (19). The LOH in *PARK2* gene was reported in primary tumor samples from patients with cervical cancer (20). The HeLa cell line, a human cervical cancer cell line, also harbors a deleted parkin gene (6) and is resistant to TNF-α induced cell death (21). In this study, we investigated the role of parkin in TNF-α-induced cell death. We report that parkin expression restores susceptibility to TNF-α-induced cell death and that this process is mediated by decreased expression of survivin and the activation of caspase-8, -9, -3, -7, and PARP.

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RESULTS

Expression of parkin in HeLa cells results in decreased cell viability after TNF-α-treatment

The parkin-deficient HeLa cell line is resistant to TNF-α-induced cell death (21). To determine whether the expression of parkin restores susceptibility to TNF-α-induced cell death, HeLa cells were infected with either mock adenovirus (Mock) or parkin-expressing adenovirus (Parkin) and then treated with different concentrations of TNF-α. As compared to Mock+TNF-α treated cells, Parkin+TNF-α treated cells showed a reduction in cell viability (Fig. 1A). The decrease in cell viability of Parkin+TNF-α treated cells was proportionate to an increase in TNF-α-concentration (Fig. 1B). No change in cell viability was observed in Mock+TNF-α treated cells at the TNF-α-concentration used reaffirming that the HeLa cell line is resistant to TNF-α. Furthermore, the M.O.I. (multiplicity of infection) of Mock used in this study exerts no adverse effects on the HeLa cells. To further confirm that the restoration of parkin resulted in susceptibility to TNF-α, we infected cells with increasing numbers of Parkin and then treated the cells with TNF-α (5 ng/ml). We found that cells infected with more Parkin were more susceptible to TNF-α effects (Fig. 1C). These results show that expression of parkin in HeLa cells restores susceptibility to TNF-α-induced cell death.

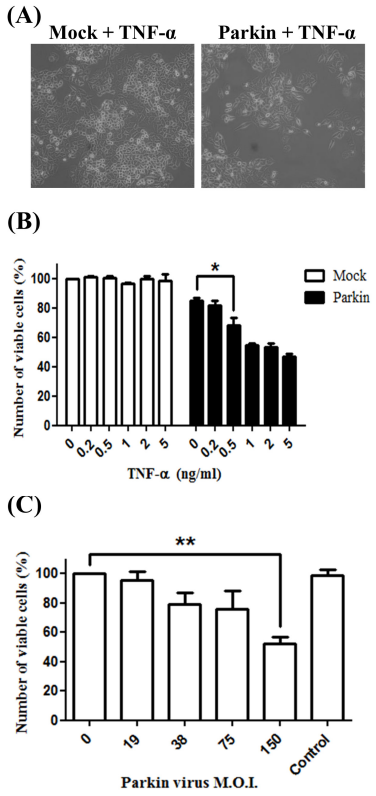

Fig. 1. Expression of parkin restores susceptibility to TNF-α-induced cell death. (A) HeLa cells were infected with either parkin-expressing adenovirus (Parkin) or mock virus (Mock) (150 M.O.I. each) for 24 h and treated with TNF-α (5 ng/ml) for 24 h. Images were captured using an inverted microscope (200×). (B) Cells were infected with either Mock or Parkin (150 M.O.I. each) for 24 h and then treated with TNF-α (0, 0.2, 0.5, 1, 2, 5 ng/ml) for 24 h. Viable cells were counted by trypan blue exclusion assay (3 independent experiments). The number of viable cells in 150 M.O.I. mock adenovirus infected group without TNF-α treatment was set as 100%. (C) Cells were infected with indicated concentrations (0, 19, 38, 75, 150 M.O.I.) of Parkin for 24 h and then treated with TNF-α (5 ng/ml) for 24 h. Viable cells were counted by trypan blue exclusion assay (3 independent experiments). The number of viable cells in the Mock (150 M.O.I.) infected group was set as 100%. Data are from three independent experiments. P values were determined by Student's *t*-test. Bars indicate SEM. *P < 0.05, **P < 0.01.

Parkin expression results in TNF-α-induced apoptotic cell death

To determine whether TNF-α-induced a decrease in cell viability in parkin expressing HeLa cells was due to apoptosis, a cell cycle analysis was performed on Parkin+TNF-α or Mock+TNF-α-treated cells by flow cytometry (Fig. 2A). The percent-

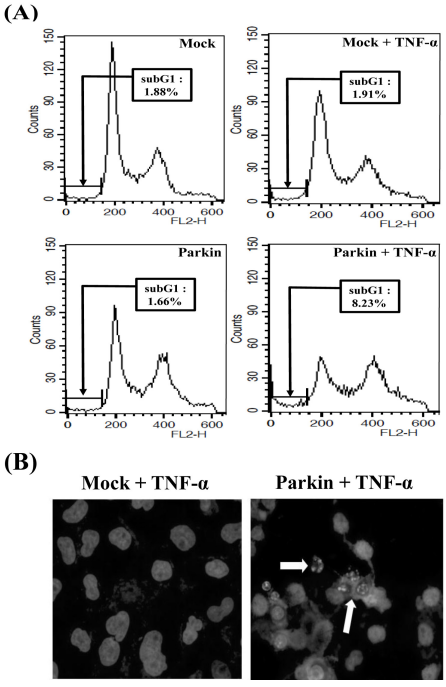

Fig. 2. Parkin enhances TNF-α-mediated cell death via activation of the apoptotic pathway. (A) HeLa cells were infected with either Parkin or Mock (150 M.O.I. each) for 24 h and then cultured with or without TNF-α (5 ng/ml) for 24 h. Cell cycle analysis was performed using propidium iodide staining and flow cytometry. (B) HeLa cells were infected with either Parkin or Mock (150 M.O.I. each) for 24 h and treated with TNF-α (5 ng/ml) for 24 h. Cells were stained with DAPI and images captured using fluorescence microscopy (430 nm). Apoptotic bodies (white arrow).

age of cells in the sub-G1 phase increased in Parkin+TNF-α treated cells (8.23%) as compared to Mock+ TNF-α treated cells (1.91%) suggesting an increase in apoptotic cells. Parkin infection alone did not increase apoptosis (1.66%) as compared to Mock infection (1.88%). Furthermore, apoptotic bodies were observed in Parkin+TNF-α treated cells but not in Mock+TNF-α treated cells using fluorescence microscopy (Fig. 2B). The increase of cells in the sub-G1 phase and the presence of apoptotic bodies in Parkin+TNF-α cells strongly suggest that parkin induces cell death in TNF-α-treated HeLa cells via the apoptotic pathway.

We investigated which proteins in the apoptotic pathway were affected in Parkin+TNF-α cells. First, we examined whether PARP, a canonical apoptotic effector protein (22), was activated in Parkin+TNF-α cells. We found that TNF-α enhanced the cleavage of the full length PARP and subsequent formation of the cleaved PARP in a Parkin dose-dependent manner (Fig. 3A, left panel). In addition, the formation of cleaved PARP increased in Parkin+TNF-α cells in a TNF-α-dose-dependent manner (Fig. 3A, right panel). Next, we determined whether caspase-3 and/or caspase-7, immediate upstream molecules of PARP, were activated in Parkin+TNF-α cells. We found that TNF-α treatment increased levels of cleaved caspase-3 and caspase-7 in Parkin+TNF-α cells in a Parkin dose-dependent manner (Fig. 3B, left panel). Similarly, the formation of caspase-3 and caspase-7 increased in Parkin+TNF-α cells in a TNF-α-dose-dependent manner (Fig. 3B, right panel). The results thus far suggest that the restoration of parkin in HeLa cells confer susceptibility to TNF-α-induced apoptosis via the activation of caspase-3, caspase-7 and PARP.

Caspase-8 and caspase-9 are upstream molecules of caspase-3/-7 (23). Caspase-8 is mainly involved in the mitochondria-independent pathway and caspase-9 is involved in the mitochondria-dependent pathway (24, 25). To determine which of these pathways are activated in Parkin+TNF-α cells, we assessed the formation of cleaved caspase-8 and cleaved caspase-9. We found increased levels of both cleaved caspase-8 and cleaved caspase-9 in Parkin+TNF-α cells in Parkin dose-dependent (Fig. 3C, left panel) and TNF-α dose-dependent manner (Fig. 3C, right panel). Taken together, our data suggest that restoration of parkin in HeLa cells confers susceptibility to TNF-α-induced apoptosis via activation of both the mitochondria-dependent and mitochondria-independent pathways.

Parkin expression results in reduced expression of survivin

In addition to caspases, a variety of proteins are involved in regulation of apoptosis (26). To identify other potential apoptotic regulatory proteins, we examined for changes in the expression of several of these molecules by RT-PCR. We detected no difference in the expression of BAK1, Bcl-xL, Bax, MCL1 and Bcl2 in Parkin+TNF-α versus Mock+TNF-α cells (Fig. 3D). However, we detected a decrease in the expression

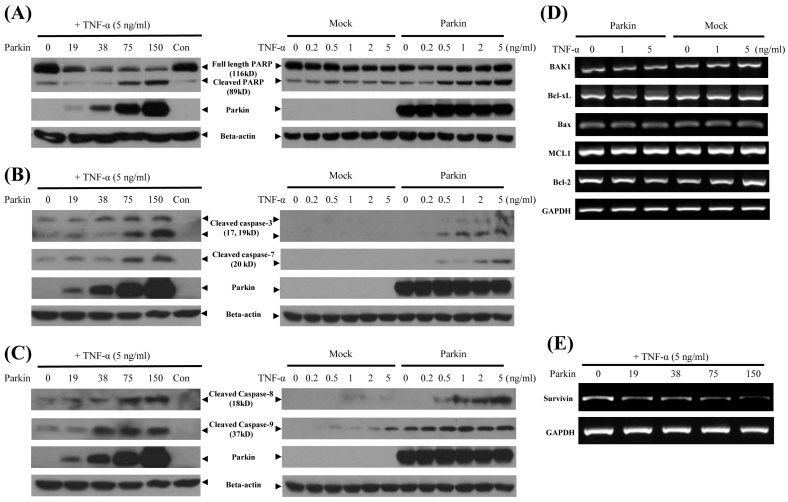

Fig. 3. Parkin expression in TNF-α-treated HeLa cells stimulates cleavage of caspases and PARP and decreases expression of survivin. Cells were infected with indicated concentrations (0, 19, 38, 75, 150 M.O.I.) of Parkin for 24 h and then treated with TNF-α (5 ng/ml) for 24 h. (A, B, C, left panels). Cells were infected with either Parkin or Mock (150 M.O.I. respectively) for 24 h and then treated with TNF-α (0, 0.2, 0.5, 1, 2, 5 ng/ml) for 24 h (A, B, C, right panels). Cleaved forms of PARP (A), caspase-3 and caspase-7 (B), caspase-8 and caspase-9 (C) were detected by Western blot. Expression of parkin was determined by Western blot using anti-parkin antibodies. Levels of β-actin were analyzed as internal controls. (D) HeLa cells were infected with either Parkin or Mock (150 M.O.I. respectively) for 24 h and then treated with indicated concentrations (0, 1, 5 ng/ml) of TNF-α for 24 h. cDNA was subjected to PCR to amplify BAK1, BCL-XL, BAX, MCL1, and BCL2. The PCR products were resolved on a 1.5% agarose gel. GAPDH was used as an internal control. (E) HeLa cells were infected with indicated concentrations (0, 19, 38, 75, 150 M.O.I.) of Parkin for 24 h and then treated with TNF-α (5 ng/ml) for 24 h. cDNA was subjected to PCR to amplify survivin. The PCR products were resolved on a 1.5% agarose gel. GAPDH was used as an internal control.

of the anti-apoptotic molecule, survivin, in a TNF-α dose-dependent manner (Fig. 3E). Since survivin is known to inhibit the activity of caspase-3 and caspase-7 (27, 28), we suggest that a decrease of survivin in Parkin+TNF-α cells is one possible mechanism by which parkin contributes to TNF-α-induced apoptosis in HeLa cells.

DISCUSSION

Although, parkin has been proposed to be a tumor suppressor, the mechanisms by which parkin functions as a tumor suppressor are unclear. In the current study, we investigated the role of parkin in TNF-α-induced apoptosis of HeLa cells. HeLa cells are inherently resistant to TNF-α-induced cell death and also lack parkin expression. We found that ***i)*** parkin expression in HeLa cells restored TNF-α-induced cell death and ***ii)*** TNF-α-induced cell death was mediated via activation of the apoptotic pathway involving survivin, caspase-8, -9, -3, -7, and PARP.

Apoptosis is induced by a variety of stimuli and one potent activator of this pathway is TNF-α (18). The lack of apoptosis due to unresponsiveness to pro-apoptotic stimuli causes uncontrolled cell proliferation leading to the development of cancers. The binding of TNF-α to tumor necrosis factor-α receptor 1 (TNFR1) has been shown to initiate the apoptosis pathway eventually leading to caspase activation (29). In the current study, we show that parkin re-expression dramatically enhances TNF-α-induced apoptosis of HeLa cells (Fig. 1B). These results strongly suggest that parkin expression in HeLa cells restores the TNF-α-dependent apoptotic pathway. A minor role of parkin in the TNF-α-independent apoptosis pathway may exist because parkin expression alone in HeLa cells lead to a ~15% reduction of viable cells. Based on these results, we suggest that a decreased expression of parkin can aid cancerous cells to escape from TNF-α-stimulated apoptosis ultimately leading to the development of cancer.

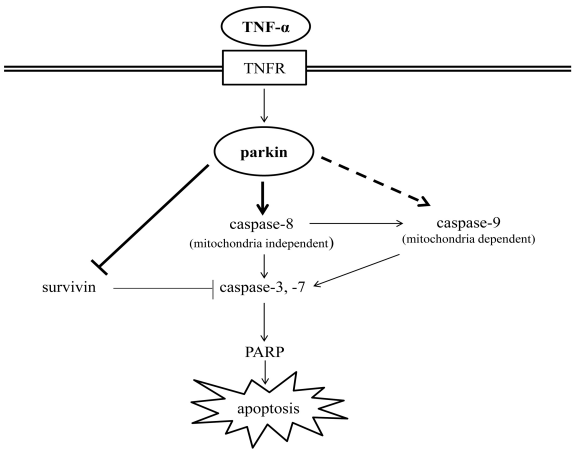

Fig. 4. Schematic model showing putative points of parkin involvement in TNF-α-induced apoptotic death of HeLa cells.

Caspases play a central role in transduction of apoptotic signals (23). There are two major apoptotic pathways; mitochondria-dependent and mitochondria-independent apoptotic pathways. Both pathways can be activated with TNF-α treatment. Caspase-8 is mainly involved in the mitochondria-independent pathway and caspase-9 is known to play an important role in the mitochondria-dependent pathway (24, 25). Our data shows that both caspase-8 and caspase-9 are activated in Parkin+TNF-α cells. Therefore, parkin is likely to exert its effect between the TNF-α receptor and prior to the initiator caspases (Fig. 4). A simpler explanation for the restoration of TNF-α induced cell death in HeLa cells could be that parkin expression restored the expression of the TNFR1 and therefore restored susceptibility to TNF-α. However, we found that HeLa cells express a stable level of TNFR1 mRNA irrespective of parkin expression (data not shown).

There are numerous pro-apoptotic molecules and anti-apoptotic proteins involved in the regulation of apoptosis (26). Among them, survivin is a member of the inhibitors of apoptosis (IAP) family. Survivin inhibits activation of caspases leading to negative regulation of apoptosis (27). Survivin binds to and inhibits the active forms of caspase-3 and caspase-7. In the present study, expression of parkin in HeLa cells reduced the expression of survivin (Fig. 3E). Therefore, the reduction of parkin in cancer cells may lead to increased levels of survivin and finally the inhibition of active caspases and ultimately to decreased apoptosis. Recent studies have shown that disruption of survivin induction pathways leads to an increase in apoptosis and decreased tumor growth (30). In our model system, a decrease of survivin by parkin may contribute to the parkin-induced restoration of TNF-α-stimulated apoptosis of HeLa cells.

Our data indicate that overexpression of parkin in HeLa cells induces cell death. However, ablation of parkin expression induced apoptotic cell death in human dopaminergic cells (31). These results suggest that in terminally differentiated cells, including neurons, reactivation of the cell cycle induces apoptosis rather than proliferation (32). Therefore, parkin may exert different effects depending on the cell type and cell cycle status. In addition, it has been proposed that parkin mediates mitophagy (33). Therefore, it would be interesting to examine whether mitophagy is associated with parkin-induced cell death in our model.

In conclusion, we report that restoration of parkin expression in the parkin-deficient HeLa cell line restored susceptibility to TNF-α-induced cell death. Although further studies are needed to determine the exact mechanisms by which parkin expression restores TNF-α-induced cell death, we have identified signaling pathways that will provide a clue to understanding how parkin acts as a tumor suppressor.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA, and trypan blue stain solution were purchased from Gibco BRL. Recombinant human TNF-α was purchased from R&D Systems. DMSO and protease inhibitor cocktail were from Sigma-Aldrich. Propidium iodide (2.5 mg/ml) was purchased from BD Biosciences. Trizol reagent, random hexamer, and MMLV-RT were purchased from Invitrogen.

Cell culture and viral infection with parkin-expressing adenovirus

HeLa (human cervical adenocarcinoma cells, ATCC) cells were infected with adenoviruses as described previously (34). In brief, cells were seeded in 6-well plates at a density of 2×10^5/well. After 24 h, cells were infected with 150 multiplicity of infection (M.O.I.) of parkin-expressing virus (Parkin) or mock virus (Mock) for 24 h. For dose-dependent experiments, cells were infected with 0, 19, 38, 75, and 150 M.O.I. of Parkin. Various concentrations of Parkin were mixed with Mock and normalized for a total of 150 M.O.I. per infection.

Trypan blue dye exclusion assay

Cells (2×10^5/well) were seeded in 6-well culture plates, cultured for 24 h and then infected with Parkin or Mock for an additional 24 h. The cells were then treated with TNF-α (5 ng/ml) for 24 h and the cells trypsinized, stained with trypan blue dye solution and viable cells counted on a hemocytometer.

DAPI staining

Cells (2×10^5/well) were seeded onto cover glass slides (24 × 24 mm) inside 6-well culture plates and cultured for 24 h. The culture medium was aspirated and the cover glass containing adherent cells was washed with PBS twice. Cells were then fixed with 2% paraformaldehyde for 5 min. After fixation, the cover glass was washed with PBS twice and then permeabilized with 0.1% Triton X-100 for 5 min. Slides were washed again and incubated with DAPI (Vector Lab, Burlingame, CA, USA) in the dark for 10 min. The cover slides were examined under an Olympus fluorescence microscope at 430 nm.

Cell cycle analysis

HeLa cells were seeded in 6-well plates (2×10^5 cells/well) and cultured in 10% FBS-DMEM for 24 h. Cells were then infected with Parkin or Mock for 24 h and then treated with TNF-α (5 ng/ml) for an additional 24 h. Thereafter, the cells were trypsinized into single cell suspension and then fixed with 70% ethanol in PBS at 4°C for 2 h. The fixed cells were stained with PBS solution containing propidium iodide (2.5 mg/ml) and RNase (0.1 mg/ml) at 37°C for 40 min. The cells were washed and the DNA content analyzed using FACS Calibur (BD Biosciences).

Western blot analysis

Cells were lysed with a PBS buffer containing 1% Triton X-100 and protease inhibitor cocktail. The supernatant was collected after centrifugation and the protein concentration determined using the Lowry protein assay (Bio-Rad). Protein samples were separated by SDS-PAGE, transferred to a nitrocellulose membrane and the membrane incubated with primary antibodies overnight and then with appropriate secondary antibodies for 1 h. Bands were visualized using ECL (Thermo, Waltham, MA, USA). β-actin was used as an internal control.

RNA extraction and reverse transcriptase PCR (RT-PCR)

Total RNA was extracted from cells using Trizol. cDNA was synthesized using 2 μg of total RNA, 0.25 μg of random hexamers and 200 U of MMLV-RT. PCR was performed using 0.2 U of *Taq* polymerase (Cosmogenetech, Korea) and specific primers (Supplemental Table 1). GAPDH was used as an internal control. PCR products were electrophoresed on 1.5% (w/v) agarose gels containing ethidium bromide and images taken using Gel Doc (Bio-Rad).

Statistical analysis

P values were calculated using Student's *t*-test. Values are shown as mean and s.e.m. Data were collected from three independent experiments.

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REFERENCES

1. Shimura, H., Schlossmacher, M. G., Hattori, N., Frosch, M. P., Trockenbacher, A., Schneider, R., Mizuno, Y., Kosik, K. S. and Selkoe, D. J. (2001) Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* **293**, 263-269.
2. Kong, F. M., Anscher, M. S., Washington, M. K., Killian, J. K. and Jirtle, R. L. (2000) M6P/IGF2R is mutated in squamous cell carcinoma of the lung. *Oncogene* **19**, 1572-1578.
3. Oates, A. J., Schumaker, L. M., Jenkins, S. B., Pearce, A. A., DaCosta, S. A., Arun, B. and Ellis, M. J. (1998) The mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R), a putative breast tumor suppressor gene. *Breast. Cancer Res. Treat.* **47**, 269-281.
4. Shridhar, V., Staub, J., Huntley, B., Cliby, W., Jenkins, R., Pass, H. I., Hartmann, L. and Smith, D. I. (1999) A novel region of deletion on chromosome 6q23.3 spanning less than 500 Kb in high grade invasive epithelial ovarian cancer. *Oncogene* **18**, 3913-3918.
5. Denison, S. R., Callahan, G., Becker, N. A., Phillips, L. A. and Smith, D. I. (2003) Characterization of FRA6E and its potential role in autosomal recessive juvenile parkinsonism and ovarian cancer. *Genes Chromosomes Cancer* **38**, 40-52.

6. Denison, S. R., Wang, F., Becker, N. A., Schule, B., Kock, N., Phillips, L. A., Klein, C. and Smith, D. I. (2003) Alterations in the common fragile site gene Parkin in ovarian and other cancers. *Oncogene* **22**, 8370-8378.
7. Poulogiannis, G., McIntyre, R. E., Dimitriadi, M., Apps, J. R., Wilson, C. H., Ichimura, K., Luo, F., Cantley, L. C., Wyllie, A. H., Adams, D. J. and Arends, M. J. (2010) PARK2 deletions occur frequently in sporadic colorectal cancer and accelerate adenoma development in Apc mutant mice. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 15145-15150.
8. Tay, S. P., Yeo, C. W., Chai, C., Chua, P. J., Tan, H. M., Ang, A. X., Yip, D. L., Sung, J. X., Tan, P. H., Bay, B. H., Wong, S. H., Tang, C., Tan, J. M. and Lim, K. L. (2010) Parkin enhances the expression of cyclin-dependent kinase 6 and negatively regulates the proliferation of breast cancer cells. *J. Biol. Chem.* **285**, 29231-29238.
9. Veeriah, S., Taylor, B. S., Meng, S., Fang, F., Yilmaz, E., Vivanco, I., Janakiraman, M., Schultz, N., Hanrahan, A. J., Pao, W., Ladanyi, M., Sander, C., Heguy, A., Holland, E. C., Paty, P. B., Mischel, P. S., Liau, L., Cloughesy, T. F., Mellinghoff, I. K., Solit, D. B. and Chan, T. A. (2010) Somatic mutations of the Parkinson's disease-associated gene PARK2 in glioblastoma and other human malignancies. *Nat. Genet.* **42**, 77-82.
10. Wang, F., Denison, S., Lai, J. P., Philips, L. A., Montoya, D., Kock, N., Schule, B., Klein, C., Shridhar, V., Roberts, L. R. and Smith, D. I. (2004) Parkin gene alterations in hepatocellular carcinoma. *Genes. Chromosomes. Cancer* **40**, 85-96.
11. Agirre, X., Roman-Gomez, J., Vazquez, I., Jimenez-Velasco, A., Garate, L., Montiel-Duarte, C., Artieda, P., Cordeu, L., Lahortiga, I., Calasanz, M. J., Heiniger, A., Torres, A., Minna, J. D. and Prosper, F. (2006) Abnormal methylation of the common PARK2 and PACRG promoter is associated with downregulation of gene expression in acute lymphoblastic leukemia and chronic myeloid leukemia. *Int. J. Cancer* **118**, 1945-1953.
12. Picchio, M. C., Martin, E. S., Cesari, R., Calin, G. A., Yendamuri, S., Kuroki, T., Pentimalli, F., Sarti, M., Yoder, K., Kaiser, L. R., Fishel, R. and Croce, C. M. (2004) Alterations of the tumor suppressor gene Parkin in non-small cell lung cancer. *Clin. Cancer Res.* **10**, 2720-2724.
13. Wang, H., Liu, B., Zhang, C., Peng, G., Liu, M., Li, D., Gu, F., Chen, Q., Dong, J. T., Fu, L. and Zhou, J. (2009) Parkin regulates paclitaxel sensitivity in breast cancer via a microtubule-dependent mechanism. *J. Pathol.* **218**, 76-85.
14. Coussens, L. M. and Werb, Z. (2002) Inflammation and cancer. *Nature* **420**, 860-867.
15. Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D. V. and Baldwin, A. S., Jr. (1998) NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* **281**, 1680-1683.
16. Beutler, B., Greenwald, D., Hulmes, J. D., Chang, M., Pan, Y. C., Mathison, J., Ulevitch, R. and Cerami, A. (1985) Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature* **316**, 552-554.
17. Aggarwal, B. B. (2003) Signalling pathways of the TNF superfamily: a double-edged sword. *Nat. Rev. Immunol.* **3**, 745-756.
18. Shin, D. H., Park, K. W., Wu, L. C. and Hong, J. W. (2011) ZAS3 promotes TNFalpha-induced apoptosis by blocking NFkappaB-activated expression of the anti-apoptotic genes TRAF1 and TRAF2. *BMB Rep.* **44**, 267-272.
19. Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E. and Forman, D. (2011) Global cancer statistics. *CA. Cancer J. Clin.* **61**, 69-90.
20. Mehdi, S. J., Alam, M. S., Batra, S. and Rizvi, M. M. (2011) Allelic loss of 6q25-27, the PARKIN tumor suppressor gene locus, in cervical carcinoma. *Med. Oncol.* **28**, 1520-1526.
21. Franco, D. L., Nojek, I. M., Molinero, L., Coso, O. A. and Costas, M. A. (2002) Osmotic stress sensitizes naturally resistant cells to TNF-alpha-induced apoptosis. *Cell Death Differ.* **9**, 1090-1098.
22. Tewari, M., Quan, L. T., O'Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S. and Dixit, V. M. (1995) Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly (ADP-ribose) polymerase. *Cell* **81**, 801-809.
23. Nunez, G., Benedict, M. A., Hu, Y. and Inohara, N. (1998) Caspases: the proteases of the apoptotic pathway. *Oncogene* **17**, 3237-3245.
24. Fernandes-Alnemri, T., Armstrong, R. C., Krebs, J., Srinivasula, S. M., Wang, L., Bullrich, F., Fritz, L. C., Trapani, J. A., Tomaselli, K. J., Litwack, G. and Alnemri, E. S. (1996) In vitro activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD- like domains. *Proc. Natl. Acad. Sci. U.S.A* **93**, 7464-7469.
25. Park, S. J., Shin, J. H., Kang, H., Hwang, J. J. and Cho, D. H. (2011) Niclosamide induces mitochondria fragmentation and promotes both apoptotic and autophagic cell death. *BMB Rep.* **44**, 517-522.
26. Igney, F. H. and Krammer, P. H. (2002) Death and anti-death: tumour resistance to apoptosis. *Nat. Rev. Cancer* **2**, 277-288.
27. Sah, N. K., Khan, Z., Khan, G. J. and Bisen, P. S. (2006) Structural, functional and therapeutic biology of survivin. *Cancer Lett.* **244**, 164-171.
28. Tamm, I., Wang, Y., Sausville, E., Scudiero, D. A., Vigna, N., Oltersdorf, T. and Reed, J. C. (1998) IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res.* **58**, 5315-5320.
29. Chen, G. and Goeddel, D. V. (2002) TNF-R1 signaling: a beautiful pathway. *Science* **296**, 1634-1635.
30. Friedrichs, B., Siegel, S., Andersen, M. H., Schmitz, N. and Zeis, M. (2006) Survivin-derived peptide epitopes and their role for induction of antitumor immunity in hematological malignancies. *Leuk. Lymphoma.* **47**, 978-985.
31. Machida, Y., Chiba, T., Takayanagi, A., Tanaka, Y., Asanuma, M., Ogawa, N., Koyama, A., Iwatsubo, T., Ito, S., Jansen, P. H., Shimizu, N., Tanaka, K., Mizuno, Y. and Hattori, N. (2005) Common anti-apoptotic roles of parkin and alpha-synuclein in human dopaminergic cells. *Biochem. Biophys. Res. Commun.* **332**, 233-240.
32. Staropoli, J. F. (2008) Tumorigenesis and neurodegeneration: two sides of the same coin? *Bioessays* **30**, 719-727.
33. Springer, W. and Kahle, P. J. (2011) Regulation of PINK1-Parkin-mediated mitophagy. *Autophagy* **7**, 266-278.
34. Kim, Y. S., Patel, S. and Lee, S. J. (2006) Lack of direct role of parkin in the steady-state level and aggregation of alpha-synuclein and the clearance of pre-formed aggregates. *Exp. Neurol.* **197**, 538-541.