Overexpression of MEKK3 Confers Resistance to Apoptosis through Activation of NFκB*

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Many cancers have constitutively activated NFκB, the elevation of which contributes to cancer cell resistance to chemotherapeutic agent-induced apoptosis. Although mitogen-activated protein kinase/extracellular-regulated kinase-3 (MEKK3) has been shown to participate in the activation of NFκB, its relations to apoptosis and cancer are unclear. In this study, we established cell model systems to examine whether stable expression of MEKK3 could lead to increased NFκB activity and confer resistance to apoptosis. In addition, we investigated in breast and ovarian cancers whether MEKK3 expression may be altered and correlated with aberrant NFκB activity. We show that stable cell lines overexpressing MEKK3 not only had elevated levels of NFκB binding activity but also were more responsive to cytokine stimulation. These stable cells showed 2–4-fold higher basal expression of Bcl-2 and XIAP than the parental cells. Consistent with this increased expression of cell survival genes, MEKK3 stable cells showed reduced activation of caspases 3 and 8 and poly(ADP-ribose) polymerase cleavage and dramatically increased resistance to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand, doxorubicin, daunorubicin, camptothecin, and paclitaxel. Intriguingly, analysis of human breast and ovarian cancers showed that a significant fraction of these samples have elevated MEKK3 protein levels with corresponding increases in NFκB binding activities. Thus, our results establish that elevated expression of MEKK3 appears to be a frequent occurrence in breast and ovarian cancers and that overexpression of MEKK3 in cells leads to increased NFκB activity and increased expression of cell survival factors and ultimately contributes to their resistance to apoptosis. As such, MEKK3 may serve as a therapeutic target to control cancer cell resistance to cytokine- or drug-induced apoptosis.

The role of mitogen-activated protein kinases (MAPKs)1 in the growth and survival of many cell types has been well
documented (1). MAPKs and stress-activated protein kinases function as mediators that regulate the cellular responses to environmental stress. They serve as key integration points along the signal transduction cascade that not only link diverse extracellular stimuli to subsequent signaling molecules but also amplify the initiating signals to ultimately activate the effector molecules and induce cell proliferation, differentiation, and survival (1, 2). The MAPK pathway participates in determining whether a cell re-enters the cell cycle or undergoes cell cycle arrest, senescence, or apoptosis (3). The MAPK/extracellular signal-regulated kinase kinases (MEKKs) are upstream regulators of the three-module cascade of MAPKs (1). MEKKs consists of a small gene family, MEKK1–6, that belongs to the serine/threonine protein kinases (1). Upon phosphorylation, MEKKs are activated and can subsequently phosphorylate and activate downstream signaling molecules, including c-Jun N-terminal kinase and p38 (2). Transient overexpression of MEKK1–3, transforming growth factor-β-activating kinase, and NFκB-activating kinase has been shown to activate NFκB (4, 5). However, except for MEKK3, genetic studies have raised uncertainties regarding their involvement in the activation of NFκB (6). In contrast, mouse embryonic fibroblasts deficient in MEKK3 were unable to activate NFκB in response to stimulation by IL-1 and TNF, indicating the functional importance of MEKK3 in the proinflammatory cytokine-mediated activation of NFκB (7).

In addition to its importance in immunity and inflammation (8), NFκB has been implicated as a major regulator that controls cancer cell survival, proliferation, and metastasis (9, 10). The transcription factor NFκB is composed primarily of hetero-meric complexes of the Rel family proteins p65 and p50. In normal resting cells, NFκB is physically confined to the cytoplasm through interaction with its protein inhibitor IκB (11, 12). Cytokines such as IL-1, tumor necrosis factor (TNF), and epidermal growth factor bind to their membrane receptors and trigger a cascade of signaling events that ultimately leads to the activation of IκB kinase, which phosphorylates IκB at two serine residues (13, 14). Phosphorylated IκB is then rapidly ubiquitinated and degraded through the 26 S proteasome pathway (15). As a result, NFκB is released from its inhibitor and translocates to the nucleus where it binds to regulatory DNA elements.

NFκB regulates the expression of many genes essential for cell growth and differentiation, such as cytokines, growth factors and their receptors, and adhesion molecules (9, 11, 12, 16, 17). It also up-regulates the expression of genes that actively participate in controlling cell survival (Bcl-2, Bcl-xl, survivin, and IAP family) (11, 16), angiogenesis (IL-8, basic fibroblast

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§ The abbreviations used are: MAPK, mitogen-activated protein kinase; NFκB, nuclear factor κB; IκB, inhibitor of NFκB; MEKK, MAPK/extracellular signal-regulated kinase kinase; IL, interleukin; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; PARP, poly(ADP-ribose) polymerase; HA, hemagglutinin; EMSA, electrophoretic mobility shift assay; HIV, human immunodeficiency virus; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DN, dominant-negative.
growth factor, and vascular endothelial growth factor) (9, 18, 19), and metastasis (matrix metalloproteases 2, 7, and 9) (20). Consistent with its regulatory role, constitutive activation of NFκB is frequently observed in different types of cancers (21) and has been correlated with cancer cell resistance to radiation- and chemotherapeutic agent-induced apoptosis (11). As such, NFκB has often been implicated in the development of tumor cell resistance to apoptosis.

As further evidence of its importance to cancer development, in some cancers, NFκB is required for oncogenic transformation (12, 22). To induce cellular transformation, many viral oncproteins activate NFκB by a variety of mechanisms (10, 11, 14). For example, the avian REV-T oncoprotein produces a constitutively active v-Rel oncprotein responsible for the progression of lymphomas and leukemias (23). The Tax oncprotein of the human T-cell leukemia virus-1 can activate the NFκB pathway by interacting directly with the IκB kinase complexes (24, 25). Inhibition of NFκB activity blocks cell transformation induced by onecgenic Ras (26) and inhibits tumor formation induced by Bcr-Abl (26, 27). In addition to viral onecgenes, abnormal expression of cellular factors also plays a major role in conferring constitutive activation of NFκB in cancer cells (28). For example, cancer cell-derived IL-1, hergulin, and/or overexpression of epidermal growth factor receptor has been shown to contribute to the constitutive activation of NFκB in breast tumors (29–31). Although recent studies showed that dysregulation of MEKK3 can affect NFκB/H9260 expression with cancer.

In this report, we investigated whether stable overexpression of MEKK3 was sufficient to lead to higher NFκB activity and increased expression of survival genes and to confer increased resistance to apoptosis. In addition, we examined whether there is altered expression of MEKK3 in breast and ovarian cancers, which might contribute to the aberrant NFκB activity. We show that stable expression of MEKK3 in cultured cells not only conferred elevated levels of activated NFκB but also induced the expression of cell survival proteins and dramatically increased their resistance to apoptosis. Further, we report for the first time that some breast and ovarian cancers have elevated expression of MEKK3 and show striking correlation between elevated MEKK3 expression and increased NFκB activity.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Reagents**—Anti-MEKK3 antibody and its secondary antibody were purchased from Upstate Biotechnology Inc. Anti-PARP antibody was obtained from BD Pharmingen. Antibodies against c-Jun N-terminal kinase, pro-caspase 3, and pro-caspase 8 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against β-actin, and α-tubulin were from Cell Signaling Technology (Beverley, MA). Anti-actin antibody was purchased from Sigma, and Bcl-2, xIAP, and pAkt were from Cell Signaling Technology (Beverley, MA). Anti-actin antibody was purchased from Upstate Biotechnology Inc. Anti-PARP antibody was purchased from Upstate Biotechnology Inc. Anti-PARP antibody was purchased from Upstate Biotechnology Inc.

**Cytotoxicity assays** were carried out following the method of Manna and Aggarwal (35). The cells (5 × 10⁴) were seeded, in triplicate, in 96-well plates and incubated with increasing concentrations of TRAIL for 72 h. The cells were then incubated with 25 µl of MTT (5 mg/ml in phosphate-buffered saline) for 2 h at 37°C. To each well, 100 µl of extraction buffer (20% SDS in 50% dimethyl formamide) was added and incubated overnight at 37°C. The absorbance was measured at 590 nm using a 96-well multiscanner autoreader (Dynatech MR.5000), with the extraction buffer as a blank. Reduction in the absorbance is indicative of cytotoxicity and reduced cell survival.

**RESULTS**

MEKK3 Cooperates with IL-1 and TNF to Activate NFκB—MEKK3 expression plasmids were cotransfected into U373 cells with a p(κB) Luciferase reporter gene. A dose-dependent increase in luciferase activity was observed, with a 9-fold in-
Overexpression of MEKK3 in Stable Cells Potentiates Cytokine-induced NFκB Activation—The transient transfection results demonstrated a direct correlation between MEKK3 levels and the ability of cells to activate NFκB in response to stimulation. To further examine this correlation, U373, Hep3B, and HEK293 cells were transfected with HA-MEKK3 expression plasmids and selected for puromycin resistance to generate U373-MEKK3, Hep3B-MEKK3, and 293-MEKK3 stable cell lines, respectively. These stable cell lines expressed elevated levels of MEKK3 as determined by the Western blots (Fig. 2A) and were used to examine the effects of higher MEKK3 expression on the activation of NFκB. All three stable cell lines showed slightly elevated constitutive NFκB binding activity; however, further stimulation with IL-1 resulted in significantly higher levels of NFκB activity than their parental cells (Fig. 2A). Consistent with the DNA binding activity, transfection of a p(κB)3/luciferase reporter also showed higher basal activity as well as greater response to IL-1 and TNF in MEKK3-stable cells than in parental cells (Fig. 2B). The synergistic induction of luciferase activity mediated by MEKK3 and IL-1 is greatly attenuated by the expression of a nondegradable IκB, either in transient cotransfection with MEKK3 expression vector or in stable cells overexpressing MEKK3 (Fig. 2C). Thus, the results from the MEKK3 stable cell lines are in agreement with the transient transfection studies and further demonstrate that MEKK3 can cooperate with IL-1- and TNF-mediated signals to synergistically activate NFκB.

MEKK3 Stable Cells Are More Resistant to TRAIL-induced Cytotoxicity—To determine whether MEKK3 plays a role in cell survival perhaps through its ability to regulate NFκB activation, U373-MEKK3 and Hep3B-MEKK3 cells and their parental cells were treated with increasing concentrations of TRAIL, and the cell viabilities were determined 72 h later by MTT assays. As shown in Fig. 3, both parental cell lines were sensitive to TRAIL, showing dose-dependent decreases in cell survival. At 0.01 nM TRAIL, ~50% of the U373 cells and greater than 80% of Hep3B cells were killed. In sharp contrast, both MEKK3 stable cell lines were resistant to TRAIL, where essentially no reduction in cell survival was observed even at 1 nM concentration (Fig. 3). These results provided strong evidence that overexpression of MEKK3 in U373 and Hep3B cells greatly increased their resistance to TRAIL-induced cytotoxicity.

MEKK3 Overexpression Blocks TRAIL-mediated Activation of the Apoptotic Pathway—Upon binding to its receptor, TRAIL induces apoptosis through the activation and processing of pro-caspases 3 and 8 to their active forms. Activated caspase 3 in turn cleaves, among other proteins, the 116-kDa PARP, ultimately resulting in apoptosis (36–38). To determine whether the reduced cell survival in TRAIL-treated parental cells was due to activation of the apoptotic pathway, U373 and U373-MEKK3 cells were treated with TRAIL for 7 h, and the cell extracts were examined by Western blot analyses for the cleavage of pro-caspases 3 and 8 and PARP. As anticipated, when treated with TRAIL, the parental U373 cells showed a dose-dependent activation of pro-caspase 3 and 8 as well as cleavage of PARP to the 85-kDa fragment (Fig. 4). At 1 nM TRAIL, a significant portion of pro-caspases 3 and 8 were processed, and a comparable fraction of PARP was cleaved. In sharp contrast, under identical conditions, U373-MEKK3 stable cells were refractory to TRAIL-induced apoptosis, showing no activation of caspases and minimal PARP cleavage. Thus, these biochemical analyses support the cell survival assays and further confirm that, when compared with parental cells, MEKK3 stable cells are highly resistant to TRAIL-induced apoptosis.
MEKK3 Stable Cells Express Elevated Levels of Cell Survival Proteins—To address the mechanism responsible for the increased resistance of stable cells to TRAIL, we examined the expression of a cell survival gene, Bcl-2 (39), and an anti-apoptotic gene, xIAP, in U373 and U373-MEKK3 cells. We reasoned that increased resistance to apoptosis might be attributed to increased expression of these genes, which are known targets of NFκB regulation (14, 40). U373 and U373-MEKK3 stable cells were treated with TRAIL, and the levels of Bcl-2 and xIAP were examined by Western blots. As shown in Fig. 5, basal expression for both Bcl-2 and xIAP expression were elevated in U373-MEKK3 cells when compared with the parental U373 cells. Treatment with TRAIL did not affect their expression in either cell line. The differential expression of Bcl-2 and xIAP between U373 and U373-MEKK3 cells was not due to protein loading differences as evidenced by the actin controls. Moreover, when these cell extracts were probed for active Akt, no difference in the levels of phospho-Akt was observed between the two cell lines, indicating that increased cell survival in MEKK3 stable cells was independent of Akt activity.

Overexpression of MEKK3 Confers Resistance to Chemotherapeutic Agents—To determine whether overexpression of
MEKK3 in stable cells may also affect their sensitivities to various chemotherapeutic agents, U373 and U373-MEKK3 cells were treated with increasing concentrations of doxorubicin, daunorubicin, camptothecin, and paclitaxel, and the cell survival was measured by MTT assays 72 h later. Although both parental and MEKK3 stable cells were killed by all four chemotherapeutic agents in a dose-dependent manner, the stable cells showed significantly greater resistance than the parental cells (Fig. 6). For example, at 1 nM doxorubicin, only 40% of the U373 parental cells survived, whereas 95% of the U373-MEKK3 stable cells survived. At a higher concentration of doxorubicin (5 μM), essentially all of the parental cells were killed, whereas 25% of the stable cells remained alive. The calculated IC50 values for these chemotherapeutic agents are presented in Table I. Taken together, our results show that in cells that have elevated expression of MEKK3, there is a corresponding increase in their resistance toward the four chemotherapeutic agents tested.

Elevated MEKK3 Levels and NFκB Activities in Breast and Ovarian Tumors—Different types of cancer have been reported to have constitutively elevated NFκB binding activity (1, 3), and many of them are resistant to apoptosis by conventional chemotherapies (47, 48). The results of our studies with the MEKK3 stable cells raise the possibility that overexpression of MEKK3 in cancer cells may be a potential mechanism that contribute to these cancer cell characteristics. To investigate whether constitutively elevated NFκB activity in cancers may, in part, be attributed to up-regulated MEKK3 expression, we examined the expression of MEKK3 in 10 human breast tumors and compared them with their matched normal breast tissues from the same patients. When cell extracts from these samples were blotted with anti-MEKK3 antibodies, we observed that although most of the breast tumors have low or undetectable MEKK3, three tumors have significantly elevated MEKK3 (Fig. 7A). In sharp contrast, MEKK3 was not detected or detected at very low levels in all 10 normal matched control tissues. Reprobing of the filter with anti-actin antibody showed equal intensities for all samples, verifying equal loading and integrity of the samples (data not shown). Nuclear extracts from several normal breast and breast tumors with high or low MEKK3 expression were prepared, and NFκB binding activities were analyzed by EMSA. As shown in Fig. 7B, breast tumors that express high levels of MEKK3 have correspondingly high NFκB binding activities, whereas tumors with low MEKK3 levels have low constitutive NFκB binding activities, comparable with those of normal control breast tissues.

To determine whether this striking correlation between increased MEKK3 expression and NFκB binding activity in breast cancers may be extended to other types of tumors, we examined MEKK3 and NFκB levels in eight ovarian tumors. Our results showed that MEKK3 was expressed at high levels in six ovarian cancer samples and at significantly lower levels in the two remaining samples (Fig. 8). Analysis of NFκB binding activity also revealed excellent correlation between MEKK3 levels and NFκB binding activities, i.e. whenever MEKK3 expression is elevated, high NFκB binding activity
was also observed. Taken together, our results show that elevated MEKK3 expression is found at high frequencies in breast and ovarian cancers. Importantly, there is a remarkable correlation between MEKK3 protein levels and NFκB binding activities in these tumors. These findings are consistent with the possibility that MEKK3 plays an important regulatory role in the constitutive activation of NFκB in breast and ovarian cancers.

**DISCUSSION**

In the present study, we sought to further define the effects of MEKK3 in the activation of NFκB and to seek a molecular explanation for the frequently observed resistance of cancer cells to cytokine- or chemotherapeutic agent-induced apoptosis. In addition, we addressed whether alterations in MEKK3 expression exist in human cancers that may be correlated with elevated NFκB activity. We established and characterized model cell systems that overexpress protein kinase MEKK3 and showed that elevated MEKK3 in these cells led to higher NFκB activities and increased expression of anti-apoptotic genes and ultimately conferred greater resistance to TRAIL- and chemotherapeutic drug-induced apoptosis. Intriguingly, we found that a significant portion of breast and ovarian cancers have elevated expression of MEKK3 with correspondingly high NFκB activity. Taken together, our results would support a role of MEKK3 in the up-regulation of NFκB activity in cancer cells.

It has been reported that in mouse embryo fibroblasts deficient for MEKK3, activation of NFκB by IL-1 and TNF was severely impaired, suggesting that MEKK3 plays an important role in the signaling pathway leading to the activation of NFκB (7). This conclusion is further strengthened in this study in

| Treatment       | IC₅₀ a (U373) | IC₅₀ a (U373-MEKK3) | Fold increase |
|-----------------|---------------|---------------------|---------------|
| TRAIL (nM)      | 1             | >50                 | >50 b         |
| Doxorubicin (μM)| 0.9           | 8                   | 8.9 c         |
| Daunorubicin (μM)| 0.6          | 25                  | 42            |
| Camptothecin (nM)| 0.0075       | 13                  | 1733 b        |
| Paclitaxel (μM)| 1             | >25                 | >25 b         |

a IC₅₀ indicates the concentration that results in 50% inhibition of cell growth.
b p < 0.001.
c p < 0.01.

**FIG. 6.** U373-MEKK3 cells are more resistant to chemotherapeutic drug-induced cytotoxicity. U373 and U373-MEKK3 cells were treated with indicated concentrations of doxorubicin, daunorubicin, camptothecin, and paclitaxel for 72 h before they were harvested for MTT assays. Cell survival values for each treatment were normalized to the values for the untreated controls, to which a value of 100% was assigned.
activation of NFκB signaling molecules activated by the cytokines. Indeed, one gesting functional cooperation between MEKK3 and other molecules.

Western blots with anti-actin antibodies were included as loading controls. NFκB binding activities as determined by EMSA. NFκB binding activity from T9 was competed with wild type (WT) or mutant (mt) oligonucleotides.

**Fig. 7. Elevated expression of MEKK3 and NFκB binding activities in breast tumors.** A, breast tumors (T) and their matched normal (N) breast tissues were homogenized, and the cell extracts were prepared to determine the levels of MEKK3 with anti-MEKK3 antibodies. B, nuclear extracts were prepared from the same breast tumors and normal breast tissues for NFκB binding activities as determined by EMSA. NFκB binding activity from T9 was competed with wild type (WT) or mutant (mt) oligonucleotides.

**Fig. 8. Elevated levels of MEKK3 and NFκB binding activities in ovarian tumors.** MEKK3 protein levels in eight ovarian tumors were determined by Western blotting (WB) with anti-MEKK3 antibodies. Western blots with anti-actin antibodies were included as loading controls. NFκB binding activities were determined by EMSA.

which transient transfection of MEKK3 or DN-MEKK3 expression plasmids resulted in a dose-dependent activation or inhibition of NFκB, respectively. These results also indicate direct participation of MEKK3 in the activation of NFκB. It is noteworthy that stimulation of MEKK3-transfected cells with cytokines resulted in dramatic synergistic activation of NFκB, suggesting functional cooperation between MEKK3 and other signaling molecules activated by the cytokines. Indeed, one such molecule downstream of IL-1 and TNF signaling pathway is Akt, which has also been reported to participate in the activation of NFκB (41–43). Consistent with this notion, we recently observed significantly higher activation of the luciferase reporter gene when MEKK3 and Akt expression plasmids were cotransfected into cells than with either one alone.7 Thus, it is likely that MEKK3 and Akt, as downstream targets of IL-1 and TNF, can function cooperatively to regulate NFκB activation. Although this is an attractive possibility, these studies do not rule out participation by other signaling molecules.

To further define the effects of MEKK3 in the regulation of NFκB and to examine the cellular consequences of elevated MEKK3 expression, we established stable cell lines in U373, Hep3B, and HEK293 cells that overexpress MEKK3. Consistent with transient transfection studies, stable expression of MEKK3 in these cells was sufficient to increase NFκB activity. Thus, these studies all showed strong correlation between MEKK3 levels and NFκB activities. Whether higher levels of MEKK3 and NFκB in these stable cells are sufficient to affect their sensitivity to cytotoxicity or survival is of great interest. We had reported earlier that although wild type mouse embryo fibroblasts are resistant to TNF, fibroblasts deficient in MEKK3 are susceptible to TNF-mediated cell cytotoxicity (7). Therefore, if MEKK3 exerts a protective effect and prevents cytokine-induced cytotoxicity, we anticipate that MEKK3 stable cells would be more resistant to cytokine-induced cytotoxicity. Indeed, such protective effects were confirmed when parental and stable cells were treated with TRAIL to induce apoptosis. Although parental U373 and Hep3B cells were readily killed by TRAIL, U373-MEKK3 and Hep3B-MEKK3 cells are resistant as determined independently by the MTT assay and cleavage of PARP and pro-caspases 3 and 8. Although the pathway(s) leading to TRAIL resistance in MEKK3 stable cells is yet to be clarified, it is likely that high levels of MEKK3 in stable cells contribute to increased activation of NFκB, which in turn up-regulates the expression of cell survival and anti-apoptotic proteins that ultimately protect the cells from apoptosis. Consistent with this hypothesis, we observed increased expression of cell survival proteins, Bcl-2 and XIAP, in U373-MEKK3 stable cells, which could protect them from TRAIL-induced apoptosis.

The role of MEKK3 in cell survival was also clearly evident in the cytotoxicity studies with four chemotherapeutic agents, doxorubicin, daunorubicin, camptothecin, and paclitaxel, commonly used to treat cancer patients (44). With all four drugs, MEKK3 stable cells exhibited considerably higher IC_{50} values than the parental cells, with paclitaxel and camptothecin showing the most dramatic increase (>25-fold, p < 0.001).

In addition to enhancing cell survival, MAPK signaling pathway also contributes in other cellular functions, including cell proliferation, cell motility, and invasion, through activation of downstream transcription factors (3, 45). As such, there is good correlation between MAPK expression and the degree of malignancy (3). Although our results showed MEKK3 stable cells acquired increased resistance to cytokine- and drug-induced apoptosis, we do not know whether they also acquired more aggressive tumor cell characteristics, such as cell proliferation, invasion, and metastasis. However, it is noteworthy that introduction of constitutively activated MAPK into melanocytes led to transformation in vivo, suggesting that expression of activated MAPK can contribute to the development of melanoma (46). Further, deregulation of MAPK activated the cell survival pathway in leukemia and solid tumors (3, 47). In another study, activated extracellular signal-regulated kinase and NFκB were able to inhibit Fas-induced apoptosis (48).

Whether overexpression of MEKK3 alone is sufficient to cause cell transformation is unknown. However, our observation that 30–40% of the human breast and ovarian cancers have significantly elevated MEKK3 levels is particularly intriguing. Moreover, many of the tumors that have elevated expression of MEKK3 also have increased constitutive NFκB DNA binding activity. It is therefore tempting to speculate that the constitutively activated NFκB in these tumors may, at least in part, be due to elevated MEKK3 activity. Future studies will expand this observation and directly examine the relationship

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*H. J. Huang and W. S.-L. Liao, unpublished data.*
between MEKK3 levels and NFκB activities in breast and ovarian cancers as well as other types of cancer. In summary, overexpression of MEKK3, either transiently or in stable cells, potentiated cytokine-mediated activation of NFκB. In the stable cells, increased MEKK3 expression is correlated with increased expression of cell survival genes Bcl-2 and xIAP and increased resistance to TRAIL- and chemotherapeutic agent-induced apoptosis. Moreover, we observed that a significant fraction of breast and ovarian cancers have aberrantly high expression of MEKK3 with concomitant elevated levels of NFκB activity. Although it is unclear at this time whether the observed high NFκB activity in cancer cells can be attributed to the high MEKK3 activity, it nevertheless raises an intriguing possibility that the levels of NFκB activation and the expression of NFκB-regulated genes involved in cancer cell survival may be controlled by specific modulation of MEKK3 activity.

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