Disruption of Hsp90 Function Results in Degradation of the Death Domain Kinase, Receptor-interacting Protein (RIP), and Blockage of Tumor Necrosis Factor-induced Nuclear Factor-κB Activation

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The death domain kinase, receptor interacting protein (RIP), is one of the major components of the tumor necrosis factor receptor 1 (TNFR1) complex and plays an essential role in tumor necrosis factor (TNF)-mediated nuclear factor κB (NF-κB) activation. The activation of NF-κB protects cells against TNF-induced apoptosis. Heat-shock proteins (Hsps) are chaperone molecules that confer protein stability and help to restore protein native folding following heat shock and other stresses. The most abundant Hsp, Hsp90, is also involved in regulating the stability and function of a number of cell-signaling molecules. Here we report that RIP is a novel Hsp90-associated kinase and that disruption of Hsp90 function by its specific inhibitor, geldanamycin (GA), selectively causes RIP degradation and the subsequent inhibition of TNF-mediated IκB kinase and NF-κB activation. MG-132, a specific proteasome inhibitor, abrogated GA-induced degradation of RIP but failed to restore the activation of IκB kinase by TNF, perhaps because, in the presence of GA and MG-132, RIP accumulated in a detergent-insoluble subcellular fraction. Most importantly, the degradation of RIP sensitizes cells to TNF-induced apoptosis. These data indicate that Hsp90 plays an important role in TNF-mediated NF-κB activation by modulating the stability and solubility of RIP. Thus, inhibition of NF-κB activation by GA may be a critical component of the anti-tumor activity of this drug.

Tumor necrosis factor (TNF)† is a proinflammatory cytokine that plays a critical role in diverse cellular events, including cell proliferation, differentiation, and apoptosis (1, 2). Many of these TNF-mediated processes can be regulated by either one of the two TNF receptors TNFR1 and TNFR2, both of which belong to the TNF/nerv growth factor receptor superfamily (3, 4). However, apoptosis is mainly induced through TNFR1, and this receptor is also known as a death receptor because it contains a death domain (5–7). TNF-induced activation of transcription factor NF-κB and c-Jun N-terminal kinase (JNK) as well as induction of apoptosis are mediated through TNFR1. The distinct signal transduction pathways that regulate these three TNFR1-mediated responses have been elucidated recently (8–11). It is known that the binding of TNF to TNFR1 leads to the trimerization of TNFR1 and the recruitment of the TNFR1-associated death domain protein (TRADD) into the receptor complex (8). TRADD subsequently serves as a platform to recruit other proteins into the complex. At least three proteins, FAS-associated death domain protein (FADD/MORT1), TNFR-associated factor 2 (TRAF2), and the receptor interacting protein (RIP), have been shown to interact directly with TRADD (8, 9, 12–16). While FADD/MORT1 is essential for TNF-induced apoptosis, the recruitment of RIP and TRAF2 are responsible for activation of NF-κB and JNK, respectively (11, 17–22). The indispensable role of RIP in TNF-induced NF-κB activation was recently demonstrated in RIP−/− mice (17). Consistent with an earlier finding that NF-κB activation protects cells from TNF-induced apoptosis, murine embryo fibroblast cells derived from RIP−/− mice are hypersensitive to apoptosis following TNF treatment (17).

Transcription factor NF-κB is composed of either homo- or heterodimers of Rel family members (23, 24). Inactive NF-κB is restricted to the cytoplasm because its interaction with inhibitory proteins termed IκBs masks its nuclear translocation signal (24). Following stimuli such as the proinflammatory cytokines TNF and interleukin-1, IκBs are phosphorylated by IκB kinase (IKK), resulting in their rapid, proteasome-dependent degradation. Recently, the IKK complex has been identified, and some subunits of this complex have been cloned (25–27). The degradation of IκBs leads to the release of NF-κB and allows NF-κB to translocate into the nucleus where it transcriptionally activates its target genes. Besides being a pivotal mediator of immune and inflammatory responses, NF-κB activation protects cells from many types of apoptosis and promotes cell survival and transformation (11, 28–32). Consistent with these observations, elevated NF-κB activity has been detected in several types of cancer (33–35).

Heat shock proteins (Hsps) are a group of chaperone proteins that help to maintain protein stability and to renature or target for degradation unfolded proteins when cells are subjected to heat shock or other stresses (36). Hsp90 is one of the most abundant Hsps, and because it regulates stability and function of a unique complement of signal transduction proteins, this chaperone is involved in a variety of important biological processes including hormone signaling, cell cycle control, and development (37–40). Hsp90 has been identified as a specific target of the novel anti-tumor drug geldanamycin (GA) (41–44). GA-induced disruption of the interaction between Hsp90 and its client proteins, including the tyrosine kinase v-Src, the
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A

| TNFα (min) | -GA | +GA | +GM |
|------------|-----|-----|-----|
| 0          | 10  | 30  | 90  |
| 30         | 60  | 120 | 0   |

IkBα

B

GST-IkBα (1-54)

kinase assay

C

GST-c-Jun (1-79)

kinase assay

D

NF-κB activity

Fold Activation

Untreated | TNFα | GA | GA + TNFα |
---------|------|----|-----------|

C-Jun transcriptional activity

Fold Activation

Untreated | TNFα | GA | GA + TNFα |
serine-threonine kinase Raf, mutant p53 proteins, and the glucocorticoid receptor, results in protein destabilization and degradation, usually mediated by the proteasome (37, 40).

Because certain TNF-mediated events have been reported to be sensitive to GA (45–47), we investigated whether disruption of Hsp90 function by GA blocks TNF-induced NF-κB activation. We report here that the death domain kinase RIP interacts with Hsp90 and that this interaction is sensitive to GA. Interruption of Hsp90/RIP association destabilizes RIP and abolishes TNF-induced NF-κB activation without affecting other TNF mediated responses, thereby increasing cellular sensitivity to TNF-induced apoptosis.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Reagents—**HeLa cells were cultured in Dulbecco’s modified Eagle’s medium high/glucone with 10% fetal bovine serum, 1 mM glutamate, 100 units/ml penicillin, and 100 μg/ml streptomycin (all purchased from Biofluids, Inc.). GA and its inactive analog geldanamycin (GM) were obtained from the Developmental Therapeutics Program, NCI (MG). IκBα was purchased from Amersham Pharmacia Biotech, baflomycin was purchased from Sigma, and cycloheximide was purchased from Calbiochem. Human TNFs was purchased from R&D Systems. N-Acetyl-leu-leu-norleucinal (LLNL) MG-115, ammonium chloride were purchased from Sigma. One ml of each extract before immune-precipitation was analyzed by SDS-PAGE to confirm the lack of effect of GA on protein general protein synthesis and stability.

**Luciferase Assay—**HeLa cells were transfected with 1 μg of the NF-κB-responsive reporter plasmid p2xNF-κB-Luc and 0.2 μg of pRSV-LacZ plasmid. As a control, 1 μg of 5× GAL4-Luc reporter plasmid, 50 ng of GAL4-c-Jun-(1–223) plasmid, and 0.2 μg of pRSV-LacZ plasmid were used to transfect HeLa cells for measuring c-Jun transcriptional activity. 24 h after transfection, cells were treated with 0.5 μM GA. After 14 h of GA treatment, cells were treated with 10 ng/ml TNFα for an additional 8 h and collected for luciferase assay as described (11). Luciferase activity was normalized according to β-galactosidase activity.

**Cell Viability Determination—**After treatment, cells were trypsinized and collected. Each sample was stained with trypan blue (Bio-Whittaker) and counted with a hemacytometer. The stained (blue) were counted as dead cells and were expressed as a percentage of total cells. 600 cells were counted for each sample. For each treatment, duplicate experiments were repeated three times.

**RESULTS**

To test whether GA blocks TNF-induced NF-κB activation, HeLa cells were treated with GA for 14 h before treatment with TNF. Because phosphorylation-dependent degradation of IκBα is essential for NF-κB activation following TNF treatment, we first examined the protein level of IκBα by Western blotting. As shown in Fig. 1A, in the absence of GA pretreatment, IκBα was degraded within 10 min of TNF addition and returned to its normal level after 1 h of treatment. In contrast, no TNF-stimulated decrease in IκBα level was detected if cells were previously treated with GA (Fig. 1A). Treatment with GM, an analog of GA that does not inhibit Hsp90 function, had no effect on the ability of TNF to stimulate the degradation of IκBα (Fig. 1A). To confirm that the signaling pathway by which TNF induces NF-κB activation is blocked by GA, we also measured the activity of IKK by in vitro kinase assay using an exogenous IκBα.

**FIG. 1.** GA blocks TNF-induced activation of NF-κB. A, HeLa cells (untreated, GA- or GM-pretreated) were incubated with 10 ng/ml human TNFα for various time periods. Incubation with GA or GM (0.5 μM) was continued for 14 h. IκBα was measured by Western blot in total lysate. GA blocked TNF-stimulated degradation of IκBα. B, HeLa cells were treated with TNFα (10 ng/ml) for 5 min, GA (0.5 μM) for 14 h, or TNFα (10 ng/ml) for 5 min after 14 h GA (0.5 μM) pretreatment. Nontreated cells were used as a control. Cells were lysed, IKK was immune-precipitated, and an immune complex kinase assay was performed to determine IKK activity using IκBα(1–54) as an exogenous substrate. GA inhibited TNF activation of IKK. C, HeLa cells were treated as described in B, and JNK activity was determined by immune complex kinase assay using GST-c-Jun(1–79) as the substrate. GA had no effect on TNF stimulation of JNK. D, TNF-induced activation of NF-κB was monitored by luciferase reporter assay. HeLa cells were transfected with the p2xNF-κB reporter (top panel) or 5xGAL4 reporter and GAL4-c-Jun plasmid (bottom panel). 24 h after transfection, some transfected cells were treated with GA (0.5 μM) for 14 h and then followed by 8 h of TNF treatment. Other transfected cells were either left as untreated or treated with TNF or GA as indicated in the figure. Luciferase assays were performed as described previously (11). Data of c-Jun transcriptional activity are the average of two independent experiments, and the results for NF-κB activity are the average of three independent experiments.
GST-IκB peptide fragment as substrate (25). Consistent with the result in Fig. 1A, GA treatment also abolished TNF-induced IKK activation because the phosphorylation of GST-IκB by IKK after TNF treatment was abolished (Fig. 1B). To determine whether GA had any effect on other TNF-induced responses, we also performed an in vitro kinase assay to examine TNF-induced JNK activation after GA treatment (using an exogenous GST-c-Jun peptide as substrate). In contrast to IKK activation, TNF-induced JNK activation was unaffected by GA (Fig. 1C). Similar observations were also made in HEK293 cells (data not shown). Finally, we monitored the effect of GA on TNF-induced NF-κB activation by studying cells transiently transfected with a NF-κB-responsive reporter plasmid. Although TNF treatment caused a 5-fold increase in reporter activity, pretreatment with GA abrogated this induction (Fig. 1D, top panel). In contrast, TNF-induced c-Jun transcriptional activity is not affected by GA treatment (Fig. 1D, bottom panel).

Although many TNF-stimulated responses can be mediated by either TNFR1 or TNFR2, in HeLa and HEK293 cells the major functional TNF receptor is TNFR1. Several TNFR1 adaptor molecules have been identified as key components of TNF signaling pathways, especially the death domain kinase RIP and the TNF receptor-associated protein TRAF2, which are responsible for TNF-dependent activation of NF-κB and JNK, respectively (11, 17–20). To investigate which step within the signal transduction pathway of TNF-induced NF-κB activation is affected by GA, we examined the protein levels of TNFR1, TRAF2, and RIP after GA treatment (14 h). Although TNFR1 and TRAF2 protein levels remained essentially unchanged, RIP protein almost completely disappeared after GA treatment (Fig. 2A). The decrease of RIP level after GA treatment is not due to masking of the epitope recognized by the monoclonal anti-RIP antibody used for Western blot analysis because identical results were obtained with a polyclonal anti-RIP antibody (data not shown). The effect of GA on RIP was both dose- and time-dependent (Figs. 2, B and C, and 4A). Furthermore, the inhibition of IKK activity correlated with the decrease of RIP protein level following GA (Fig. 2C).

Because GA destabilizes several tyrosine and serine-threonine kinases including v-Src and the mitogen-activated protein kinase kinase Raf (41, 48), we tested whether the decrease of RIP level in the presence of GA was due to RIP destabilization. HeLa cells were incubated with either GA or cycloheximide for 14 h (CHX, used as a control for general protein synthesis inhibition), and protein levels of RIP, TRAF2, and Myc were measured. As shown in Fig. 3A, whereas GA treatment decreased RIP level, CHX treatment had no effect, suggesting that GA-mediated RIP depletion was due to enhanced RIP instability. In contrast, although GA had no effect on Myc levels, CHX caused the disappearance of Myc protein, which is known to have a short half-life (Fig. 3A). Neither GA nor CHX had any effect on TRAF2 (Fig. 3A). We also performed reverse transcription-PCR experiments with two RIP-specific primers to check RIP mRNA levels and found no difference before or after GA treatment (data not shown). These data suggested a specific effect of GA on RIP protein stability. To confirm the hypothesis that GA destabilizes RIP, we measured RIP protein half-life by $^{35}$S methionine/ $^{35}$S cysteine pulse-chase analysis in the presence and absence of GA (Fig. 3B). Exposure of cells to GA clearly resulted in a markedly decreased RIP protein half-life. In control cells, a true half-life could not be determined, even when the chase period was extended to 8 h (data not shown), whereas in GA-treated cells the half-life of RIP protein was estimated to be 2.5 h.

The kinases v-Src and Raf form a stable complex with Hsp90, and GA destabilizes v-Src and Raf proteins by interfering with Hsp90 function (41, 48). To test whether RIP interacts with Hsp90 and to examine the possible effects of GA on this interaction, we immune-precipitated RIP and analyzed resultant blots for Hsp90 and Hsp80-associated co-chaperone proteins. Because we only detected a small decrease in RIP protein level 6 h after exposure to GA (Fig. 4A), we performed RIP immunoprecipitation assays at 0, 2, and 6 h after GA treatment. As shown in Fig. 4B (left panel), Hsp90 co-precipitated with RIP in untreated cells and GA-treated cells. As a control, anti-HA antibody was used to perform the immune-precipitation experiment with untreated cell extract, and no Hsp90 was pulled down (Fig. 4B, right panel). Because GA disrupts Hsp90 function by preventing recruitment of the co-chaperone p23 into an Hsp90 complex while instead favoring Hsp90 association with Hsc70 (51), we examined whether such changes could be observed in the composition of RIP-Hsp90 complexes following GA treatment. By sequentially blotting the same membrane shown in Fig. 4B (left panel) with anti-Hsc70 and anti-p23 monoclonal antibodies, we found that although the Hsc70 content of RIP immune-precipitates increased by 6 h after GA treatment, the presence of p23 in this complex dramatically decreased, despite comparable amounts of RIP precipitated in each assay. The total level of Hsp90, p23, and Hsc70 in these
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Fig. 3. Geldanamycin treatment shortens RIP protein half-life. A, HeLa cells were incubated with either 0.5 μM GA or 10 μg/ml CHX for 14 h, and RIP, TRAF2, and Myc protein levels were determined by Western blotting. B, RIP protein stability was determined in the presence and absence of GA by [35S]methionine pulse-chase analysis, as described under “Experimental Procedures.”

Fig. 4. RIP association with Hsp90 is altered by geldanamycin. A, HeLa cells were treated with 0.5 μM GA for the indicated time periods. RIP and TRAF2 protein levels were measured by Western blotting. B, HeLa cells were treated as in A, and protein extracts from those cells were collected in TNE5 buffer and then immediate-precipitated (IP) with anti-RIP antibody. Input is 1% of the extract from untreated cells. Resultant blots were analyzed for co-precipitated Hsp90, Hsc70, p23, and RIP proteins. Anti-HA antibody was used as an antibody control.

Fig. 5. Geldanamycin-stimulated RIP degradation is mediated by the proteasome. A, HeLa cells were treated with the proteasome inhibitor MG-132 (25 μM) 1 h before and during overnight treatment with 0.5 μM GA. Cells were collected in 60 μl Laemmli buffer and subjected to Western blotting analysis for RIP content. B, cells treated as in A were collected in TNE5 buffer, and the presence of RIP protein in the detergent-soluble lysate fraction was monitored by Western blotting with anti-RIP antibody (left panel). In the right panel, cells were treated with 10 ng/ml TNFa for 20 min without pretreatment of 25 μM MG-132. The detergent-soluble lysate fragment was then analyzed for IκBα protein content by Western blotting with anti-IκBα antibody. C, in the left panel, HeLa cells were treated with the lysosome inhibitor bafilomycin (BF; 0.1 μM) 1 h before and during overnight treatment with GA. Cells were collected in Laemmli buffer and processed for RIP determination as in A. In the right panel, HEK293 cells were exposed to several protease inhibitors for 1 h before addition of GA (1 μM), and cells were lysed in TNE5 buffer 14 h after GA addition. Equal protein (50 μg) from Nonidet P-40-insoluble fractions was analyzed for RIP content. The final concentration and specificity of the protease inhibitors used was as follows: N-acetyl-leu-leu-norleucinal (LLnL, proteasome), 100 μM; chloroquine (CQ), lysosome, 200 μM; MG-132 and MG-115 (proteasome), 10 μM; NH4Cl (lysosome), 20 mM; benzoyloxycarbonyl-Val-Ala-Asp (ZVAD) (caspase), 50 μM.

The presence of MG-132 antagonized GA-stimulated RIP degradation. As a control for MG-132 efficiency in this experiment, its effect on TNF-induced IκBα degradation was examined by Western blotting cell lysate with anti-IκBα antibody. MG-132 completely blocked TNF-stimulated degradation of IκBα (Fig. 5B, right panel). Interestingly, when cells were collected in a buffer containing nonionic detergent and only a soluble protein extract was used for Western blotting, we failed to detect RIP recovery from GA treatment when MG-132 was added (Fig. 5B, left panel). This suggested that RIP became insoluble when its interaction with Hsp90 was disrupted by GA. To confirm the insolubility of RIP in the presence of GA and the proteasome dependence of GA-stimulated RIP degradation, we examined RIP levels in a detergent-insoluble cell extract following treatment of cells with GA and a number of protease inhibitors (Fig. 5C). The proteasome inhibitors N-acetyl-leu-leu-norleucinal, MG-132, and MG-115 all protected RIP from GA-stimulated degradation but caused the kinase to appear in the detergent-insoluble fraction, whereas three lysosome inhibitors, chloroquine (CQ), ammonium chloride, and bafilomycin as well as the caspase inhibitor benzoyloxycarbonyl-Val-Ala-Asp (ZVAD) were unable to protect RIP from GA (Fig. 5C). None of the protease inhibitors tested had any effect in themselves on either RIP levels or RIP solubility (Fig. 5C and data not shown).

These results indicate that GA inhibits TNF-induced NF-κB activation subsequent to the destabilization of RIP and stimulation of its proteasome-dependent degradation. Next, we asked whether prevention of RIP degradation by MG-132 was
The uniform expression level of HA-IKK in TNF-induced IKK activation (seventh through eighth lanes), whereas MG-132 failed to reverse GA inhibition of IKK stimulation by TNF (fifth through sixth lanes). As a control, the presence of MG-132 alone had minimal effect on IKK activation (seventh through eighth lanes).

Together with our earlier data, these results suggest that interaction of RIP and Hsp90 is necessary to maintain both the stability and solubility of RIP before its recruitment to the TNF receptor and that RIP/Hsp90 association is essential for RIP to transduce a TNF signal. Because RIP is rapidly recruited from the cytoplasm into a TNFR1 complex following TNF treatment (10), we wished to know whether TNF treatment has any effect on RIP association with Hsp90. After treating HeLa cells with TNF for varying periods of time, we evaluated RIP and Hsp90 interaction by performing immune-precipitation with anti-RIP antibody and probing the resultant blots with anti-Hsp90 antibody (Fig. 6B, top panel). Whereas Hsp90 co-precipitated with RIP in untreated cells, as we have shown above, interaction between RIP and Hsp90 was greatly decreased within 5 min of TNF treatment, although similar amounts of RIP were immune-precipitated in each case (Fig. 6B, bottom panel). These data indicate that TNF treatment induces the rapid release of RIP from Hsp90. The interaction of RIP and Hsp90 reappeared gradually between 15 and 30 min after TNF treatment (Fig. 6B, top panel).

Since activation of NF-κB protects cells against TNF-induced apoptosis as well as against many other types of cell death, it may play a critical role in tumorigenesis. To determine the functional consequences of GA-dependent abrogation of NF-κB activation by TNF, we investigated whether GA can sensitize cells to TNF-induced apoptosis. HeLa cells were treated with either GA, TNF, or TNF plus GA. As demonstrated in Fig. 7, although GA or TNF alone only killed 4 and 7 percent of cells, respectively, the combination of GA and TNF caused nearly 35 percent of cells to undergo apoptosis within this time period. In contrast, GM does not have the same effect of GA (Fig. 7). These data strongly suggest that GA can potentiate TNF-induced cell death.

**DISCUSSION**

TNF imparts information to cells through a number of signal transduction cascades (2). It is known that TNF-induced NF-κB activation protects cells from undergoing apoptosis (53). Several earlier reports have described a putative but uncharacterized GA-sensitive component in certain aspects of TNF signaling (45–47), leading us to undertake the current study. Our present results indicate that GA selectively blocks TNF-induced NF-κB activation without affecting TNF-induced JNK activation, thereby sensitizing cells to TNF-induced apoptosis.

**Fig. 7. Geldanamycin enhances the apoptotic effects of TNF.** Two dishes of HeLa cells were pretreated with 0.5 µg/ml GA for 8 h, and the other two were untreated. Then one dish of cells from each group was treated with 10 ng/ml TNFα for 12 h. During TNF treatment, GA was not removed. As a control, GM was used to perform the identical experiments as described with GA. Cells were trypsinized and collected in PBS buffer. The viability of cells was determined by trypan blue exclusion assay. Each bar represents the average of three independent experiments. Data are normalized to the rate of spontaneous cell death occurring in untreated cells (less than 5%).
A specific antagonist of the chaperone protein Hsp90, inhibits TNF-induced NF-κB activation by interfering with the interaction of RIP and Hsp90, resulting in RIP destabilization and its subsequent proteasome-mediated degradation.

GA has recently been shown to bind specifically and with high affinity to Hsp90 (42–44). Hsp90 bound to GA cannot recruit the co-chaperone protein p23, resulting in an Hsp90 multichaperone complex lacking p23 but containing elevated levels of Hsc70 (54). These Hsp90 complexes cannot stabilize Hsp90 client proteins in a functional conformation, resulting instead in their rapid destabilization and degradation, frequently mediated by the proteasome (40). Our current data thus identify RIP as a novel cytosolic protein kinase that requires association with an Hsp90/p23-containing chaperone complex to maintain its stability. Interestingly, blocking GA-induced RIP degradation with the proteasome inhibitor MG-132 failed to restore TNF-induced NF-κB activation, presumably because the RIP protein so protected remains insoluble in the absence of a productive association with Hsp90. Therefore, Hsp90, by maintaining RIP solubility as well as stability, may provide multiple levels of regulation of TNF signaling.

Hsp90 is now known to play an important role in many signal transduction networks (37, 39, 40). The chaperone, in concert with a host of co-chaperone protein partners, stabilizes its client proteins while keeping them in a conformation able to respond to appropriate stimuli. At least in the case of steroid receptors, Hsp90 dissociates from the receptor upon ligand binding (55). Similarly, our data demonstrate that TNF stimulates rapid dissociation of Hsp90 from RIP, concomitant with its presumed recruitment to the TNF receptor complex. Taken together, our observations suggest a third function for RIP/Hsp90 association, namely to permit signal-dependent translocation of the kinase from cytosol to the TNF receptor complex at the plasma membrane. A similar role for Hsp90 has been proposed for the Ras-dependent trafficking of Raf kinase from cytosol to plasma membrane (56, 57). Raf translocation has also been shown to be disrupted by GA (48).

Previously, an Hsp90 homologue, TRAP-1, has been identified as a TNF receptor-1-interacting protein that may play a role in TNF signaling (58). We initially expected that GA, by interfering with TRAP-1, might affect TNF signaling at the level of the TNF receptor. In fact, GA has recently been shown to bind to TRAP-1, albeit with a 10-fold weaker affinity than to Hsp90 (59). However, because the TNF receptor level remains unchanged after GA treatment and because TNF-induced JNK activation is not altered by the drug, it is unlikely that the abrogation of NF-κB activation described in this report is mediated by GA interference at the level of the TNF receptor. Nonetheless, it is still possible that TRAP-1 plays an important role in regulating other aspects of TNF signaling. If so, it will be intriguing to understand how Hsp90 and TRAP-1 cooperate to modulate TNF activity.

Several growth-promoting kinases have been described to be Hsp90 client proteins (40), and inhibition of their function may partially account for the antitumorogenic properties of GA (39). Here we report that GA, by destabilizing RIP, abrogates TNF-induced NF-κB activation and sensitizes HeLa cells to TNF-induced apoptosis. To begin to examine the general physiologic significance of this observation, we compared the cytoxicity of TNF and GA alone with that of the two agents combined toward the TNF receptor-expressing breast cancer cell line MCF7, and we obtained similar results as in HeLa cells. Because NF-κB activation has been found to be essential for cell transformation and tumor proliferation (29), blocking of NF-κB activation by GA may provide an additional and important rationale for use of GA as an anti-tumor agent. Since many anti-cancer chemotherapeutics also activate NF-κB, resulting in self-limitation of their own cytotoxicity (35), it will be interesting to investigate whether GA can inhibit NF-κB activation in this way. Although the mechanisms through which anti-cancer drugs activate NF-κB are unknown, it is possible that chemotherapy-induced up-regulation of members of the TNF family may be involved. For example, doxorubicin and methotrexate induce the transcription of Fas ligand in human T-cell leukemia cell lines (60). In these cases, GA may prove to be an ideal adjuvant when combined with such chemotherapeutic agents.

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