Inhibition of the Mitotic Kinesin Eg5 Up-regulates Hsp70 through the Phosphatidylinositol 3-Kinase/Akt Pathway in Multiple Myeloma Cells*

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The microtubule-dependent motor protein Eg5 plays a critical role in spindle assembly and maintenance in mitosis. Herein we show that the suppression of Eg5 by a specific inhibitor arrested mitosis, induced apoptosis, and up-regulated Hsp70 in human multiple myeloma cells. Mechanistically, Hsp70 induction occurred at the transcriptional level via a cis-regulatory DNA element in Hsp70 promoter and was mediated by the phosphatidylinositol 3-kinase/Akt pathway. Eg5 inhibitor-mediated Hsp70 up-regulation is cytotoxic because blocking Hsp70 induction directly by antisense or small interfering RNA or indirectly by inhibiting the phosphatidylinositol 3-kinase/Akt pathway significantly increased Eg5 inhibitor-induced apoptosis. Furthermore, a farnesyltransferase inhibitor interacted synergistically with the Eg5 inhibitor in inducing apoptosis through disrupting the Akt/Hsp70 signaling axis. These findings provide the first evidence for Eg5 inhibitor activity in hematologic malignancy and identify Hsp70 up-regulation as a critical mechanism responsible for modulating myeloma cell sensitivity to Eg5 inhibitors. In addition, these findings suggest that a combination of Eg5 inhibitors with agents abrogating Hsp70 induction would be useful for myeloma therapy in the clinic.

Inhibitors of Eg5 (also named kinesin spindle protein or kinesin-5) have emerged as a new class of agents that interfere with the mitotic spindle (7). Eg5 is a microtubule-dependent motor protein of the kinesin family that is crucial in mitosis for spindle assembly and maintenance (8–16). The first small-molecule inhibitor of Eg5, monastrol, was discovered in a phenotype-based screening and so named because it gave rise to cells with monopolar spindles (monoasters) (10). Since then, a number of Eg5 inhibitors have been identified, including HR22C16, CK1016023, EG5-IA, and dimethylenastron (17–20). Dimethylenastron is a member of the quinazoline-2(1H)-thione class and is identified in an in vitro ATPase assay-based screening as a specific, cell-permeable Eg5 inhibitor (20). Similar to microtubule inhibitors, Eg5 inhibitors arrest mitosis and trigger apoptosis. Because Eg5 is not expressed in postmitotic neurons and is likely to act only in dividing cells (18), its inhibitors might provide better specificity than microtubule inhibitors in the treatment of human malignancies.

Eg5 inhibitors have shown promising anticancer activity in preclinical studies and are currently being investigated in phase I and phase II clinical trials. The Eg5 inhibitor dimethylenastron has shown antiproliferative activity in human cervical carcinoma cells (20). In this study, we examined if and how dimethylenastron exerts a therapeutic function in human multiple myeloma cells. We found that dimethylenastron halted cell cycle progression in mitosis and induced apoptosis. Dimethylenastron activated the PI3K/Akt pathway, which in turn caused a remarkable transcriptional up-regulation of heat shock protein 70 (Hsp70). Dimethylenastron-induced apoptosis was enhanced by blocking Hsp70 induction directly with antisense or small interfering RNA or indirectly by inhibiting the phosphatidylinositol 3-kinase (PI3K)/Akt pathway with pharmacologic or dominant-negative approaches. Furthermore, we found that FTI277, a specific small-molecule inhibitor of farnesyltransferase, synergized with dimethylenastron in inducing apoptosis by interrupting the PI3K/Akt/Hsp70 cascade. These results provide the first evidence for the effectiveness of Eg5 inhibitors in killing human multiple myeloma cells and uncover critical molecular events that regulate myeloma cell sensitivity to Eg5 inhibitors.

**EXPERIMENTAL PROCEDURES**

*MATERIALS—Dimethylenastron was prepared as described previously (20), and LY294002, LY303511, and FTI277 were obtained from Calbiochem.

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chem. Antibodies against α-tubulin and β-actin were from Sigma, antibodies against Hsp70 were from Stressgen, and all the other primary antibodies were from Cell Signaling. Horseradish peroxidase-conjugated anti-rabbit and anti-mouse secondary antibodies were purchased from Amersham Biosciences, and fluorescein-conjugated anti-mouse secondary antibodies were from Jackson ImmunoResearch Laboratories.

### Cells and Viruses
Human multiple myeloma RPMI8226 cells were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO₂. Adenoviruses encoding Akt, dominant-negative Akt, Hsp70, and antisense Hsp70 and retroviruses encoding Hsp70 small hairpin interfering RNA were prepared and amplified in low passage human embryonic kidney 293 cells as described previously (21–24).

### Cytotoxicity Assay
Cells grown in 96-well plates were treated with gradient concentrations of dimethylenastron for 48 h. Sulforhodamine B assay was then performed to evaluate drug cytotoxicity and to determine the drug concentration needed for 50% cell killing (LD₅₀).

### Analysis of Combined Drug Effects
Cells were treated with a range of dimethylenastron and FTI277 concentrations alone and in combination at a fixed ratio of 1:10 for 48 h. At the end of this period, the extent of cell death was measured by a cytotoxicity assay for each condition. Treatment interaction effects between dimethylenastron and FTI277 were then determined by calculating the combination index (CI) values for each fraction affected using the commercially available CalcuSyn program (Biosoft), which is based on the principle of Chou and Talalay (25). CI values less than 1 correspond to synergistic drug interactions, CI values equal to 1 correspond to additive interactions, and CI values more than 1 correspond to antagonistic interactions.

### Flow Cytometry
Flow cytometric evaluation of cell cycle status was performed as described previously (26, 27). Briefly, 2 × 10⁶ cells were collected, washed twice with ice-cold phosphate-buffered saline (PBS), and fixed in 70% ethanol for 24 h. Cells were washed again with PBS and incubated with 20 μg/ml propidium iodide and 20 μg/ml RNase A in PBS for 30 min in dark. Samples were analyzed on a Coulter Elite flow cytometer (Beckman). Annexin V staining assay was performed by using the annexin V apoptosis detection kit (BD Biosciences). Briefly, cells were washed with PBS and then resuspended in binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂). Cells were incubated with fluorescein-conjugated annexin V for 15 min in the dark, suspended in binding buffer, and then analyzed on the flow cytometer as described above.

### Immunofluorescence Microscopy
Cells grown on polylysine/fibronectin-coated glass coverslips were fixed with methanol for 5 min at −20 °C, washed with PBS, and blocked with 2% bovine serum albumin in PBS. Cells were incubated with a mouse monoclonal anti-α-tubulin antibody (Sigma) and then a fluorescein-conjugated secondary antibody followed by staining with propidium iodide for 10 min. Coverslips were mounted with 90% glycerol in PBS and examined with a Zeiss fluorescence microscope.

### Luciferase Reporter Assay
Cells were transfected with pRL-TK Renilla luciferase reporter plasmid (Promega) and Hsp70 promoter...
driven firefly luciferase reporter plasmids (28). Luciferase activities were then determined by using the dual luciferase reporter assay system according to the manufacturer’s protocol (Promega).

**FIGURE 2. Dimethylenastron up-regulates Hsp70 expression.** A, Western blot analysis of Hsp70 and β-actin in cells treated with 1 μM dimethylenastron for 0, 6, 12, 18, 24, or 30 h. Hsp70 protein levels were determined by densitometric analysis of the protein bands on Western blots and normalized to the measured value at 0 h of treatment. B, Western blot analysis of Hsp70 and β-actin in cells treated with the indicated concentration of dimethylenastron for 12 h. Hsp70 levels were determined by densitometry as in A. DIYDEN, dimethylenastron. C, cells were transfected with the Hsp70 promoter-driven firefly luciferase reporter plasmid pHsp70(–259) and the thymidine kinase promoter-driven Renilla luciferase reporter plasmid pRL-TK for 24 h and then treated with 1 μM dimethylenastron for 0, 6, 12, 18, 24, or 30 h. The pHsp70(–259) luciferase activity was normalized to the Renilla luciferase activity and is shown here in arbitrary unit. D, cells were transfected with pRL-TK and a series of luciferase reporter plasmids driven by the Hsp70 promoter of different length and treated with 1 μM dimethylenastron for 12 h. Luciferase activities were determined as in C.

**FIGURE 3. Involvement of the PI3K/Akt pathway in dimethylenastron-mediated Hsp70 up-regulation.** A, Western blot analysis of phosphorylated Akt (p-Akt), p38, ERK, p-ERK, and JNK (p-JNK) as well as total Akt, p38, ERK, and JNK in RPMI8226 cells treated with 1 μM dimethylenastron for 1, 2, 4, 8, or 12 h. The level of Akt phosphorylation is defined as the phosphorylated Akt level divided by the Akt level followed by normalization to the value at 0 h of treatment. B, cells were treated with 1 μM dimethylenastron for 6 h with 1 μM dimethylenastron in the absence or presence of the PI3K inhibitor LY294002 (20 μM) or the inactive control compound LYO3351 (20 μM). The levels of Akt phosphorylation and Hsp70 were then determined by densitometry. DIYDEN, dimethylenastron. C, cells were transfected with the pHsp70(–259) and pRL-TK luciferase reporter plasmids for 24 h and treated for 12 h with 1 μM dimethylenastron in the absence or presence of LY294002 (20 μM) or LYO3351 (20 μM). Luciferase activities were then determined using the dual luciferase reporter assay. D, cells were transfected with the pHsp70(–259) and pRL-TK luciferase reporter plasmids for 12 h, infected with control adenovirus (5 m.o.i.) or dominant-negative Akt (DN-Akt) adenovirus (5 or 25 m.o.i.) for another 12 h, and then treated with 1 μM dimethylenastron before the determination of luciferase activities. The level of Akt phosphorylation was examined by Western blot analysis as in A.

**RESULTS**

**Eg5 Inhibitor Dimethylenastron Induces Mitotic Arrest and Apoptosis in Human Multiple Myeloma Cells—**Using a sulforhodamine B-based cytotoxicity assay, we found that dimethylenastron was highly potent against RPMI8226 human multiple myeloma cells with an LD₅₀ of 0.48 μM (Fig. 1A). Like other known Eg5 inhibitors, dimethylenastron blocked cell cycle progression in mitosis and induced monoannular spindles (Fig. 1, B and C). Prolonged treatment of cells with dimethylenastron induced apoptosis, as revealed by a significant increase in the number of cells with sub-G₁ (hypodiploid) DNA content (Fig. 1B), annexin V positive staining (Fig. 1D), and cleavage of caspase-3 and poly(ADP-ribose) polymerase (Fig. 1E).

**Dimethylenastron Up-regulates Hsp70 at the Transcriptional Level in Myeloma Cells—**Our two-dimensional polyacrylamide gel electrophoresis initiative using extracts from dimethylenastron-treated and untreated RPMI8226 cells identified Hsp70 as a protein up-regulated by dimethylenastron, which was confirmed by Western blot analysis (Fig. 2, A and B). Hsp70 protein level was induced by 12-fold at 6 h of di
methyleneastron treatment and by 17-fold at 12 h of treatment. Surprisingly, longer treatment decreased the effect of dimethylenastron on Hsp70 up-regulation (Fig. 2A). We also examined the level of Hsp70 after cells were treated with different dimethylenastron concentrations, and found that dimethylenastron up-regulated Hsp70 in a dose-dependent manner (Fig. 2B).

There are several reports indicating that Hsp70 can be induced by heat shock and mitogenic agents primarily at the transcriptional level (31, 32). To examine whether dimethylenastron also regulates Hsp70 transcriptionally, we performed a luciferase reporter assay using pHsp70(K85) (28). Consistent with the results obtained from Western blot analysis, dimethylenastron treatment increased the pHsp70(K85) luciferase activity (Fig. 2C). Interestingly, although Hsp70 protein induction peaked at 12 h of drug treatment (Fig. 2A), maximal induction of pHsp70(K85) luciferase activity occurred at 18 h post-treatment (Fig. 2C).

To further characterize the transcriptional regulation of Hsp70 by dimethylenastron, we transfected cells with a series of luciferase reporter plasmids driven by different sizes of Hsp70 promoters, including pHsp70(−259), pHsp70(−200), pHsp70(−163), pHsp70(−123), and pHsp70(−82), which differed in size because of 5′ deletions (28). Dimethylenastron induced comparable luciferase activities in pHsp70(−259), pHsp70(−200), and pHsp70(−163) but significantly reduced activities in pHsp70(−123) and pHsp70(−82) (Fig. 2D). These results indicate that the sequence between −163 and −123 in the Hsp70 promoter contained the cis-regulatory DNA element that mediates Hsp70 transcriptional up-regulation by dimethylenastron.

**The PI3K/Akt Pathway Plays a Role in Dimethylenastron-mediated Up-regulation of Hsp70—** The PI3K/Akt pathway and the pathways of the mitogen-activated protein kinases, i.e. p38, extracellular signal-regulated kinase (ERK), and c-Jun amino-terminal kinase (JNK), have been implicated in the up-regulation of Hsp70 (33–38). To determine whether these pathways play a role in the up-regulation of Hsp70 by
FIGURE 6. Synergistic interaction between the FTI277 and the Eg5 inhibitor dimethylenastron in inducing apoptosis in myeloma cells. A, cells were treated with varying concentrations of dimethylenastron and FTI277 alone and in combination at a fixed ratio of 1:10 for 48 h. At the end of this period, the extent of cell death was measured by a cytotoxicity assay for each condition. Treatment interaction effects between dimethylenastron and FTI277 were then determined by calculating the CI values for each fraction affected using the CalcuSyn program, based on the principle of Chou and Talalay (25). CI < 1, synergistic drug interaction; CI = 1, additive drug interaction; CI > 1, antagonistic drug interaction.
dimethylenastron, the activation of Akt, p38, ERK, and JNK was examined by Western blot analysis using antibodies against phosphorylated (activated) forms of these proteins (Fig. 3A). We found that dimethylenastron rapidly activated Akt with maximal activation at 4 h post-treatment. In contrast, there was no detectable activation of p38, ERK, or JNK (Fig. 3A). Furthermore, dimethylenastron-induced Akt activation was inhibited by LY294002, a potent and specific inhibitor of PI3K, but not by LY303511, an inactive analog of LY294002 (Fig. 3B). More importantly, dimethylenastron-induced Hsp70 up-regulation was largely inhibited by LY294002, but not by LY303511 (Fig. 3B), clearly implicating the PI3K/Akt pathway in dimethylenastron-mediated Hsp70 up-regulation.

The role of PI3K/Akt pathway in Hsp70 induction was further investigated by using the luciferase reporter assay. We found that dimethylenastron-mediated Hsp70 promoter activity was significantly attenuated by the PI3K inhibitor (Fig. 3C). In addition, adenovirus-mediated expression of a dominant-negative Akt inhibited dimethylenastron-induced Hsp70 promoter activity in a dose-dependent manner (Fig. 3D). Together, these results indicate that the PI3K/Akt pathway plays a role in dimethylenastron-induced Hsp70 up-regulation.

**Inhibition of Hsp70 Induction Sensitizes Cells to Dimethylenastron-induced Apoptosis**—Hsp70 has an anti-apoptotic effect through various mechanisms. For example, Hsp70 can directly bind and antagonize the function of several pro-apoptotic proteins, such as apoptosis-inducing factor (AIF), Apaf-1, and apoptosis signal-regulating kinase 1 (ASK1) (39–43). We investigated whether Hsp70 induction exerts an anti-apoptotic effect in response to dimethylenastron treatment. As shown in Fig. 4A, adenoviruses encoding Hsp70-specific antisense RNA reduced Hsp70 expression in dimethylenastron-treated cells and promoted dimethylenastron-induced apoptosis, whereas control adenoviruses had no obvious effect. Similarly, retroviral expression of small interfering RNA against Hsp70 (Hsp70si) also potently inhibited dimethylenastron-induced Hsp70 up-regulation (Fig. 4B). As expected, knockdown of Hsp70 significantly sensitized cells to dimethylenastron-induced apoptosis (Fig. 4C).

We also examined whether inhibition of Hsp70 induction by blocking the PI3K/Akt pathway also enhances dimethylenastron-induced apoptosis. As shown in Fig. 5A, inhibition of Akt phosphorylation by the PI3K inhibitor LY294002 significantly enhanced dimethylenastron-induced apoptosis at a concentration of 25 μM, whereas its effect was only subtle at 1 or 5 μM. The enhancing effect of PI3K/Akt inhibition on dimethylenastron-induced apoptosis was also observed when dominant-negative Akt was used (Fig. 5B). Moreover, the presence of excessive wild-type Akt could prevent the effect of dominant-negative Akt (Fig. 5B). Together, these results suggest that Hsp70 induction plays a cytoprotective role in response to dimethylenastron, and abrogating Hsp70 induction could potentiate dimethylenastron-induced apoptosis either directly or indirectly.

**FTI277 Induces Apoptosis Synergistically with Dimethylenastron through Disrupting the PI3K/Akt/Hsp70 Cascade**—Farnesyltransferase inhibitors (FTIs) represent a new generation of chemotherapeutic drugs currently undergoing clinical evaluation (44). They inhibit the farnesylation of H-Ras, Rheb, RhoB, CENP-E, CENP-F, and several other cellular proteins and thereby interfere with a number of signaling pathways, including the PI3K/Akt pathway (45). Because the PI3K/Akt pathway mediates dimethylenastron-induced up-regulation of Hsp70, it is possible that FTIs might sensitize cells to dimethylenastron-induced apoptosis by interrupting the PI3K/Akt/Hsp70 cascade. We examined this possibility by treating cells with varying concentrations of dimethylenastron and FTI277 (a specific FTI) alone and in combination at a fixed ratio of 1:10 for 48 h. At the end of this period, the extent of cell death was measured by a cytotoxicity assay for each condition. Treatment interaction effects between dimethylenastron and FTI277 were then determined by calculating the CI values for each fraction affected using the CalcuSyn program, based on the principle of Chou and Talalay (25). Such analysis yielded CI values less than 1 for the combination of dimethylenastron with FTI277, corresponding to a synergistic interaction between these two drugs (Fig. 6A).

Annexin V staining assay further revealed that 1 μM FTI277 did not cause obvious apoptosis but substantially potentiated dimethylenastron-induced apoptosis (Fig. 6B). Western blot analysis showed that FTI277 markedly inhibited dimethylenastron-induced Akt activation and Hsp70 induction (Fig. 6C), suggesting that the synergistic interaction between FTI277 and dimethylenastron is mediated by the Akt/Hsp70 pathway. Our data further showed that inhibition of Hsp70 synthesis by FTI277 decreased long term (72 h) survival of dimethylenastron-treated cells (Fig. 6D). In addition, we found that adenovirus-mediated overexpression of Akt or Hsp70 protected cells from dimethylenastron/FTI277-induced apoptosis, and the level of protection provided by delivery of Akt was accompanied by a similar level of Hsp70 expression as when Hsp70 was delivered (Fig. 6, E and F). These results thus indicate that FTI277 synergizes with dimethylenastron in inducing apoptosis through interfering with the PI3K/Akt/Hsp70 signaling axis.

We then examined whether overexpression of Hsp70 in cells not treated with FTI277 could increase the survival of dimethylenastron-treated cells. Short term measures (36 h) of apoptosis revealed that Hsp70 overexpression inhibited dimethylenastron-induced apoptosis (Fig. 6G). Furthermore, long term (72 h) measures of viability revealed that Hsp70 overexpression increased the survival of dimethylenastron-treated cells (Fig. 6H). These results show the relevance of Hsp70 to long term cell survival in dimethylenastron-treated cells.

**DISCUSSION**

Multiple myeloma, also known as myeloma or plasma cell myeloma, is the second most prevalent hematologic malignancy after non-Hodgkin lymphoma. Chemotherapy is one of the major treatment options to today’s myeloma patients. Unfortunately, clinical use of currently available drugs is hampered by various side effects. Thus, there is an urgent need to identify agents with higher specificity and fewer side effects to improve the treatment of this disease. Over the past years, small-molecule inhibitors of the mitotic kinesin Eg5 have demonstrated potential as novel chemotherapeutics. However, their therapeutic value in hematologic cancer has not been examined. In the present study, we show for the first time that dimethylenastron, an Eg5-specific cell-permeable inhibitor, potently inhibited cell proliferation and induced apoptosis in myeloma cells. We also demonstrated that dimethylenastron...
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inhibits myeloma cell proliferation by blocking mitosis, resulting in cells with monopolar spindles. This phenotype is similar to that observed when cells are injected with Eg5 antibodies or treated with other Eg5 inhibitors (8, 9, 12, 17–19, 46) and is consistent with the role of Eg5 in bipolar spindle assembly. Cells treated with dimethylenastron, similar to those treated with other Eg5 inhibitors and other anti-spindle agents, eventually succumb to apoptosis following mitotic arrest. These results indicate that Eg5 could be a valuable therapeutic target, and dimethylenastron could be useful in the treatment of myeloma.

Regarding the mechanism of action of Eg5 inhibitors, previous studies were largely limited to the inhibition of mitotic progression and induction of apoptosis. In the present study, we demonstrated that the Eg5 inhibitor dimethylenastron up-regulated Hsp70 protein in a time- and dose-dependent manner. Hsp70 induction occurred transcriptionally as indicated by an increase in Hsp70 promoter activity. Although Hsp70 promoter activity peaked at 18 h post-dimethylenastron treatment, Hsp70 protein induction peaked at 12 h. Other mechanisms could thus be involved in the regulation of Hsp70 by dimethylenastron. We also found that dimethylenastron activated Akt, but not p38, ERK, or JNK, prior to the induction of Hsp70. In addition, inhibition of the PI3K/Akt pathway significantly blocked Hsp70 induction. These results indicate that the PI3K/Akt pathway is crucial for dimethylenastron-mediated Hsp70 up-regulation. Nevertheless, there was a modest induction of Hsp70 protein and Hsp70 promoter activity even in the presence of high concentrations of PI3K inhibitors or dominant-negative Akt adenoviruses. Therefore, although the PI3K/Akt pathway plays an important role in dimethylenastron-induced Hsp70 expression, other regulatory pathways may also exist.

By serial deletion analysis, we have identified a cis-regulatory sequence in Hsp70 promoter, located between nucleotides −163 and −123, as the major region responsible for dimethylenastron-induced Hsp70 up-regulation. This region is upstream to the heat shock element and the binding sites for signal transducers and activators of transcription (32, 47). A Web-based navigation through transcription factor binding data bases, using the dimethylenastron response sequence as bait, does not provide instructive information as to which known transcription factor(s) might be involved in the up-regulation of Hsp70 by the Eg5 inhibitor.

Hsp70 up-regulation is known to occur as part of the cellular defense system against environmental stresses such as heat shock, oxidative stress, or the treatment with chemical agents (48, 49). Consistent with these findings, we found that Hsp70 up-regulation plays a cytoprotective role in response to the Eg5 inhibitor dimethylenastron, as blocking Hsp70 induction either directly by expressing antisense RNA/small interfering RNA or indirectly by inhibiting the PI3K/Akt pathway dramatically increased dimethylenastron-induced apoptosis. Although Hsp70 induction may involve different mechanisms under different circumstances, its ability to promote cell survival is likely related to its anti-apoptotic effect per se. It is well established that Hsp70 regulates pro-apoptotic proteins negatively to inhibit apoptosis. For example, Hsp70 can directly bind to AIF to antagonize AIF-dependent apoptosis (42). Hsp70 also binds to Apaf-1 to prevent the recruitment of caspases to the apoptosome complex and thereby to block the apoptotic signaling relay (40, 41). In addition, Hsp70 physically associates with ASK1 to block its homo-oligomerization, inhibiting apoptotic cell death (43).

Our results also reveal Hsp70 up-regulation as a critical mechanism modulating myeloma cell sensitivity to Eg5 inhibitors.

On the other hand, our findings indicate that abrogating Hsp70 induction is important in increasing Eg5 inhibitor sensitivity. Furthermore, we found that the FTI277 interacted synergistically with the Eg5 inhibitor dimethylenastron to induce apoptosis in myeloma cells. Importantly, FTI277 interruption of dimethylenastron-induced Akt/Hsp70 signaling cascade underlies directly the synergistic combination of these two agents. These results suggest that a combination of Eg5 inhibitors with agents abrogating Hsp70 induction like FTIs may be more useful in myeloma therapy.

In summary, we have shown that inhibition of the mitotic kinesin Eg5 by a specific inhibitor leads to mitotic arrest, apoptosis, and Hsp70 up-regulation in human multiple myeloma cells. Hsp70 induction appears to be an early event and is mediated by the PI3K/Akt pathway. In addition, Hsp70 up-regulation has been found to play a cytoprotective role, and disruption of the Akt/Hsp70 signaling axis can greatly potentiate Eg5 inhibitor-induced apoptosis. These findings offer the first evidence for Eg5 inhibitor activity in hematologic malignancy and provide novel insights into the mechanisms underlying Eg5 inhibitor sensitivity. Our results also suggest that a combination of Eg5 inhibitors with agents abrogating Hsp70 induction is a promising approach in treating multiple myeloma for which no curative therapies exist to date.

Acknowledgments—We thank Dick Mosser, Allan Brasier, Kenneth Walsh, and Michael Sherman for providing reagents. We are also grateful to Dick Mosser and Allan Brasier for their critical comments on the manuscript.

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