Modulation of the c-Met/Hepatocyte Growth Factor Pathway in Small Cell Lung Cancer

Gautam Maulik, Takashi Kijima, Patrick C. Ma, Sudip K. Ghosh, Jeffrey Lin, Geoffrey I. Shapiro, Erik Schaefer, Elena Tibaldi, Bruce E. Johnson, and Ravi Salgia

Departments of Adult Oncology [G. M., T. K., P. C. M., J. L., G. I. S., B. E. J., R. S.] and Immunobiology [E. T.], Dana-Farber Cancer Institute, and Department of Medicine, Brigham and Women’s Hospital, and Harvard Medical School, Boston, Massachusetts 02115; Biosource International, Hopkinton, Massachusetts 01748 [E. S.]; and Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal 721302, India [S. K. G.]

ABSTRACT

The c-Met receptor tyrosine kinase and its ligand HGF (hepatocyte growth factor) have been shown to be involved in angiogenesis, cellular motility, growth, invasion, and differentiation. The role of c-Met/HGF axis in small cell lung cancer (SCLC) has not been reported previously. We have determined the expression of p170c-Met precursor and p140c-Met β-chain in seven SCLC cell lines by immunoblotting. We used the SCLC cell line H69, which expressed an abundant amount of c-Met to study the function and downstream effects of c-Met activation. Stimulation of H69 cells with HGF (40 ng/ml, 6-h stimulation) significantly altered cell motility of the SCLC cells with increased formation of filopodia and membrane ruffling, characterized as membrane blebbing, as well as increased migration of the cellular clusters were seen. We have further studied the signal transduction pathways of HGF/c-Met in the H69 cell line. The stimulation of H69 with HGF (40 ng/ml, >24 h) increased the amount of reactive oxygen species formed by 34%. HGF stimulation (40 ng/ml, 7.5-min stimulation) of H69 cells showed increased tyrosine phosphorylated bands identified at M 68,000, 120,000–140,000, and 200,000. Some of these tyrosine-phosphorylated bands were identified as the focal adhesion proteins paxillin, FAK, PYK2, and the c-Met receptor itself. Phospho-specific antibodies show that the focal adhesion proteins paxillin, FAK, PYK2, and the c-Met receptor tyrosine kinase are phosphorylated in response to HGF/c-Met signal transduction. We also demonstrate that the Hsp90 inhibitor geldanamycin, which also affects c-Met, reduced the growth and viability of four of four SCLC cell lines by 25% to 85%, over a 72-h time period. Geldanamycin caused apoptosis of SCLC cells, as well as led to increased levels of Hsp70 but not Hsp90. These results demonstrate that c-Met/HGF pathway is functional in SCLC, and it would be useful to target this pathway toward novel therapy.

INTRODUCTION

SCLC is an aggressive cancer that tends to metastasize relatively quickly. Even with chemotherapeutics available for the past 30 years, long-term survival rates have been quite dismal (1). New and novel therapeutics are being tested in patients with SCLC to arrive at better control of disease with the goal of longer survival. Recently, tyrosine kinase receptors have been implicated in the etiology of multiple tumors, and they may be important therapeutic targets (2). We have reported recently that the tyrosine kinase c-Kit is an important RTK in SCLC that is targeted by the tyrosine kinase inhibitor STI 571 (3). This has been applied clinically, and STI 571 has been introduced into a Phase II clinical trial for patients with previously untreated SCLC and patients with relapsed sensitive disease.

c-Kit tyrosine kinase activation by stem cell factor is only one of many mechanisms proposed for the pathogenesis of SCLC. As shown previously, c-Met RTK mRNA is also expressed in lung cancer cells (4). c-Met is an RTK, which stimulates the invasive growth of carcinoma cells, is tumorigenic, and overexpressed in many solid tumors (5). The c-Met receptor is a disulfide linked α-β heterodimer with a molecular weight of M 190,000 (6). The M 140,000 β-chain spans the membrane and possesses cytoplasmic tyrosine kinase activity and can be detected in its precursor form at M 170,000. The natural ligand for c-Met is the HGF (also known as scatter factor; Refs. 7 and 8). HGF stimulation of c-Met can lead to proliferation, increased survival, altered motility, enhanced invasion into extracellular matrix, and more rapid formation of tubules (9). c-Met overexpression, as well as activating mutations in the various domains, can lead to carcinogenesis in multiple tumors. There are multiple activating mutations in the c-Met gene identified in hereditary papillary renal carcinomas (10). c-Met, on activation by autophosphorylation, can associate with and activate multiple signal transducing intermediates, such as Grb2, p85 subunit of PI3k, Stat-3, and Gab1 (9).

The abbreviations used are: SCLC, small cell lung cancer; RTK, receptor tyrosine kinase; HGF, hepatocyte growth factor; ROS, reactive oxygen species; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; DCF-DA, dichloro-fluorescein-diacetate; DMTU, 1,3-dimethylthiourea; PI3k, phosphatidylinositol 3'-kinase; WCL, whole cell lysate; IP, immunoprecipitated lysates.
In lung cancer, c-Met mRNA levels can be elevated. As an example, in a study of 25 SCLC cell lines, it was shown that 22 of 25 cell lines express c-Met mRNA and only 2 of 25 cell lines coexpressed HGF mRNA (4). c-Met appears to be activated in a paracrine fashion, with HGF being produced by the stromal cells. c-Met has shown to be expressed in the non-small cell lung cancer cell line A431, and activation by HGF stimulates cell growth, scattering, and invasion of these cells (4). HGF levels in tumor tissue have been directly associated with shortened survival in patients with non-small cell lung cancer (11). In an effort to determine the role of c-Met in SCLC, we show by immunoblotting that c-Met protein is expressed and phosphorylated in SCLC, as well as functional through the cytoskeleton. Patterns of phosphoregulation of individual tyrosine residues were determined for cytoskeletal proteins in response to HGF in SCLC, including p125FAK, PYK2, and paxillin. We further show that targeting of c-Met by geldanamycin reduced phosphorylation of signaling proteins, inhibited growth and viability in SCLC cells, and altered the levels of the molecular chaperone Hsp70.

MATERIALS AND METHODS

Cell Lines and Cell Culture. The SCLC cell lines were obtained from the American Type Culture Collection (Rockville, MD). The SCLC cell lines were maintained in RPMI 1640 (Cellgro), 10% (volume for volume) FCS, and 1% (volume for volume) penicillin-streptomycin (12). All cell lines were incubated at 37°C with 5% CO2. The cell lines were harvested during log phase growth. For stimulation studies with HGF, cells were deprived of growth factors by incubating the cells without FCS for 16 h, and the media comprised of 0.5% BSA (Sigma Chemical Co., St. Louis, MO). The SCLC cell lines that expressed c-Met were tested for protein tyrosine phosphorylation in response to HGF at the dose of 40 ng/ml for 7.5 min. This short-time incubation was shown to be the time for maximal phosphorylation in a kinetic analysis (data not shown). H69 cells were incubated with 40 ng/ml HGF for ≤6 h to observe the formation of filopodia, change in membrane ruffling, and migrational movement of the clusters by time-lapse video microscopy. For ROS levels, we determined over 24 h since late oxidative species can form during this time. For ROS, H69 cells were treated with or without HGF (40 ng/ml).

MTT Assays. Cell viability was measured by MTT colorimetric dye reduction assay (Sigma Chemical Co.; Ref. 13). Each data point was repeated in independent experiments and performed in quadruplicates, and SDs were calculated. Geldanamycin was obtained from the National Cancer Institute and used to determine the effects on SCLC cell growth and viability by the MTT assay (14).

Antibodies. The antiphosphotyrosine monoclonal antibody 4G10 was obtained from UBI (Lake Placid, NY). Anti-c-Met antibody (C-12), anti-Hsp90 antibody, anti-Hsp70 antibody, anti-p125FAK antibody, and anti-PYK2 antibody were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and were used per manufacturer’s directions. Antiphospho antibody (clone 5H11) was used as described previously (15). Polyclonal phosphorylation site-specific antibodies (Biosource International, Camarillo, CA) to tyrosine 31 or tyrosine 118, both known CRKL SH2 domain binding sites, or tyrosine 181 in paxillin; tyrosine 397 (autophosphorylation site) and tyrosine 861 (Src phosphorylation Site) in p125FAK; and tyrosine 402 (autophosphorylation site) and 881 (Grb2 binding site) in PYK2 were used.

Preparation of Cell Lysates and Immunoblotting. Cell lines were lysed in lysis buffer as described previously (15). Cell lysates were separated by 7.5% SDS-PAGE under reducing conditions, electrophoretically transferred to pure nitrocellulose transfer and immobilization membrane (Schleicher & Schuell, Keene, NH), and processed for immunoblotting using established methods with enhanced chemiluminescence technique (NEN Life Science Products, Boston, MA). Also immunoprecipitations were performed according to standard procedures and immunoblotting thereafter (16).

Analysis of ROS. A total of 106 cells were treated with or without 5 μM DCF-DA (2′, 7′-dichlorofluorescein-diacetate; Acros Organics, Pittsburgh, PA) for 7.5 min at 37°C and subsequently washed twice in cold Dulbecco’s PBS before analysis using a Coulter Epics XL flow cytometer (Coulter Corp., Miami, FL). The fluorescence of oxidized DCF was measured with an excitation wavelength of 480 nm and an emission wavelength of 525 nm (17).

Cell Cycle Analysis and Apoptosis Detection by Flow Cytometry. SCLC cells were treated at 37°C with either DMSO or geldanamycin at concentration of 100 nM and analyzed after propidium iodide staining using standard methods (3). Apoptosis detection by flow cytometry was performed using the Apoptosis Detection System, Fluorescein (Promega, Madison, WI), based on the TUNEL assay, as per the manufacturer’s directions.

Time-lapse Video Microscopy. Serum-starved cells were placed in uncoated plastic tissue culture plates (35 × 10 mm plates; Becton Dickinson Labware), and after 6 h of observation, they were treated with 40 ng/ml HGF in a temperature-controlled chamber at 37°C in standard media. The cells were examined by video microscopy using Olympus IX70 inverted microscope, omega temperature controlled device, Optronics Engineering DEI-750 video camera, Olympus OEV142 TV, and Panasonic AG6740 time-lapse S-VHS video recorder continuously. The digital video images were then processed with an Apple computer containing a G4 microprocessor and analyzed with the NIH Image Analysis program.

Confocal Microscopy. F-actin was visualized in fixed cells (18). FITC-phalloidin (Sigma Chemical Co.) was used to determine F-actin. Confocal image analysis was performed using a Leica NT-TCS confocal microscope fitted with argon and krypton lasers.

RESULTS

Expression of c-Met in SCLC Cell Lines. A polyclonal antibody against c-Met was used to detect the p170c-Met precursor expression, as well as the p140c-Met β-chain (Fig. 1A). In the H69 and H345 SCLC cell lines, the p170 precursor and p140 forms were strongly detected. There was moderate expression of both in H128 and H146 SCLC cell lines, and in H82, H209, and H446 SCLC cell lines, only p140c-Met was detected by immunoblotting. In H82 and H128 cell lines, p140c-Met expression...
appeared as a doublet, and H209 and H446 expressed a slightly larger form of p140 c-Met. Four of the SCLC cell lines were tested for tyrosine phosphorylation of proteins in response to HGF (40 ng/ml; Fig. 1B). There was increased tyrosine phosphorylation of multiple proteins in three of four cell lines used, with the most dramatic tyrosine phosphorylation change in H69 cell line. Increased tyrosine phosphorylation was visualized at Mr 200,000, 170,000, 140,000, 120,000, 90,000, 70,000, 60,000, and 40,000 in response to HGF.

**c-Met Tyrosine Phosphorylation in Response to HGF in H69 SCLC Cell Line.** Because there was a robust expression of c-Met in H69 SCLC cell line, as compared with very low expression of c-Met in H209 cell line, we determined tyrosine phosphorylation of c-Met in response to HGF in these lines. As seen in Fig. 2A, with anti-c-Met immunoprecipitation and phosphotyrosine immunoblot, there was increased tyrosine phosphorylation of c-Met in response to HGF (7.5 min, 40 ng/ml). The amount of c-Met immunoprecipitation is the same with or without HGF (Fig. 2B). In converse experiment, phosphotyrosine immunoprecipitation and anti-c-Met immunoblot revealed the same result (Fig. 2C).

**Altered Cell Motility in H69 SCLC Cell Line.** The above data show that c-Met is functionally active in SCLC. We have used the H69 cell line as a model, because there was sufficient expression of c-Met to determine the impact of adding HGF to other functions in SCLC.

As shown previously (3), H69 SCLC cells characteristically move together as a cluster and display membrane ruffling, characterized by membrane blebs (Fig. 3A). When SCLC cells are separated from the cluster, they either unite with another group of cells or, if isolated, they undergo apoptosis (data not shown). With HGF treatment (40 ng/ml) of the SCLC, the cells had dramatically increased membrane ruffling and formation of filopodia, as well as increased migrational movement of the cellular clusters (Fig. 3B). As calculated by NIH Image Analysis program, the average size of filopodia formed with HGF stimulation was 4 ± 1.2 (SD) μm.

F-actin staining of SCLC cells treated with HGF also
HGF Leads to an Increase in ROS. We analyzed the endogenous levels of ROS in H69 cell line, with and without treatment of HGF (40 ng/ml) over 24 h (Fig. 5) using 2',7'-DCF-DA by flow-cytometry. The relative ROS levels were increased in response to HGF by ~30%. DCF-DA can measure oxidizing agents, such as hydrogen peroxide, superoxide anion, and hydroxyl radicals (17). Using DMTU, which is a potent scavenger of hydroxyl radicals, the ROS levels decreased in response to HGF. Without HGF treatment and DMTU, there was no further decrease in ROS levels.

HGF Stimulation of SCLC Cells Leads to Phosphorylation of Multiple Cytoskeletal Proteins. HGF activates cytoskeletal proteins leading to altered cell motility. We looked for activation of specific pathways known to be involved in the regulation of the focal adhesion. Phosphorylation of cytoskeletal proteins p125FAK, PYK2, and paxillin was determined by using a panel of phosphorylation site-specific antibodies. We determined the activation of specific phosphorylation sites in these proteins, known to be important in the regulation of cytoskeletal functions. As shown in Fig. 6, p125FAK was phosphorylated on tyrosines 397 (autophosphorylation site) and 861 (the major Src phosphorylation site), in response to HGF. Similarly, PYK2 was phosphorylated on tyrosines 402 (autophosphorylation site) and 881 (Grb2 binding site) in response to HGF. Finally, HGF treatment resulted in higher migrating forms of paxillin coinciding with phosphorylation at tyrosine 31 (the first CRKL binding site) but not tyrosines 118 or 181.

Geldanamycin Inhibits Cell Growth of SCLC Cells. Geldanamycin is an antibiotic with multiple effects on tumor cells and has been shown to disrupt the c-Met/HGF axis (14). We have determined the effects of geldanamycin on growth and viability, cell cycle, cell motility, and signal transduction of SCLC cells.

Cell viability curves in Fig. 7 show that over 72 h of treatment with geldanamycin (~200 nM), there was decreased viability seen in the four SCLC cell lines tested (H69, H82, H146, and H249). The calculated IC₅₀ values are: 89 nM for H69, 54 nM for H146, and 86 nM for H249 cell lines. At the concentrations used, 50% decrease in cell viability was not seen in H82 cell line.

Using the H69 cell line, cell cycle analysis was performed (Fig. 8, A–D) with or without HGF and geldanamycin. In response to 100 nM geldanamycin, there was an increase (from 14 to 34%, no HGF treatment; from 15 to 32%, with HGF treatment) in the sub-G₁ population. The sub-G₁ population corresponded to apoptotic events because an independent TUNEL assay with geldanamycin of H69 cells confirmed this finding (Fig. 8E). We also detected decreased cell migration, formation of filopodia, and formation of membrane ruffles within 2–4 h of geldanamycin treatment (data not shown). HGF...
stimulation of cells did not enhance or abrogate the effects of geldanamycin.

**Geldanamycin Regulates c-Met Function and Expression.** We next investigated the effect of geldanamycin on downstream tyrosine phosphorylation after the addition of HGF. In H69 cells treated with geldanamycin, there was a decrease in tyrosine phosphorylation of proteins. Additionally, although the amount of protein on the gel is similar, there was a decreased amount of c-Met in response to geldanamycin treatment. Because geldanamycin directly inhibits the function of the molecular chaperone Hsp90, the effects on the levels of c-Met with Hsp90 and its related chaperone Hsp70 were examined. In dose-responsive fashion, there were increased levels of Hsp70 and not Hsp90. By cross-immunoprecipitation studies, we did not appreciate any association between Hsp70 or Hsp90 with c-Met itself (data not shown).

**DISCUSSION**

The studies of RTKs in solid tumors have exploded recently because of the potential utility of these molecules as molecularly targeted therapies (2). In studies shown here, we have documented that HGF/c-Met pathway is functional in SCLC, and this may be a useful target for therapeutic intervention in SCLC. We have shown in this study that c-Met was expressed in SCLC cell lines; downstream tyrosine phosphorylation of proteins (with $M_r$ 220,000, 110,000–140,000, and 90,000) occurred in a short time interval, as well as in a dose-responsive fashion (data not shown) in response to HGF. c-Met expression was heterogeneous in the various different SCLC cell lines, and it is possible there may be mutations of the c-Met gene or overamplification of the gene in certain SCLC cell lines. This possibility is...
currently being investigated. Stimulation of c-Met by HGF leads to increased formation of ROS and increased cell motility. This is of special interest because stromal cells in the lung are known to produce HGF and thereby have the potential to activate c-Met in lung cancer cells.

We have shown here that cell motility of SCLC is enhanced by the addition of HGF. The mechanism whereby HGF stimulation of c-Met leads to increased motility, migration, and invasion in cancer cells has not been well worked out. c-Met stimulation promotes cell movement, causes epithelial cells to disperse (“scatter”) and endothelial cells to migrate, and promotes chemotaxis (20–23). Invasion is also important in c-Met signaling because mutant mice nullizygous for Met show that muscles originating from dermomyotome cells that migrate to the limb, diaphragm, and tip of the tongue fail to develop (24).

In this study, c-Met stimulation as reflected by tyrosine phosphorylation by HGF leads to increased migration, membrane ruffling, and filopodia formation in SCLC cells. It would now be interesting to determine which phosphorylation sites of c-Met are activated by HGF in SCLC. Therefore, it is quite possible that activation of the c-Met signaling pathway contributes to the metastatic mechanism of SCLC.

Cell motility is also regulated by the focal adhesion. In suspension cells, such as SCLC, the focal adhesions are re-

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**Fig. 8** Cell cycle analysis and apoptosis assay of H69 cells with geldanamycin treatment (A–D). Untreated (A and C) or HGF-treated (B and D; 24 h, 40 ng/ml) H69 SCLC cells with (C and D; 24 h, 100 nM) or without (A and B) geldanamycin. SCLC cells were fixed and stained with propidium iodide for flow cytometry. E, apoptosis detection using the TUNEL assay shown without or with geldanamycin (24 h, 100 nM).

**Fig. 9** HGF-stimulated tyrosine phosphorylation in SCLC can be inhibited by treatment with geldanamycin. A, expression of tyrosine phosphorylated proteins. Antiphosphotyrosine immunoblot of control or geldanamycin-treated cells. There were decreased tyrosine phosphorylation bands (M, 120,000–150,000) in response to HGF (7.5 min, 40 ng/ml) in the geldanamycin-treated cells (100 nM, 24 h). B, c-Met expression. The same membrane was stripped and probed for anti-c-Met. c-Met expression appears to be decreased in the geldanamycin-treated lanes. C, PI3k expression. Control PI3k immunoblot was done of the same membrane, showing similar loading of proteins.

**Fig. 10** Geldanamycin treatment alters the levels of Hsp70 and not Hsp90. H69 cells were treated with geldanamycin with various concentrations (0, 100, and 1000 nM) for 24 h in starved medium. The lysates were prepared according to “Materials and Methods.” Immunoblots of the same membrane were performed in a sequential fashion, using anti-c-Met, anti-Hsp90, anti-Hsp70, and anti-β-actin antibodies.
flected as punctuate focal contacts (25). We have shown that certain components of the focal adhesion, such as Paxillin, p125Fak, and PYK2, are phosphorylated at specific sites in response to HGF. Induction of phosphorylation of FAK and PYK2 at their autophosphorylation sites indicates that the initial activation step has occurred. The fact that the GR2 binding site, on PYK2, becomes phosphorylated in response to HGF suggests a means to differentially activate the Ras/mitogen-activated protein kinase pathway. Interestingly, HGF has been known for some time to activate calcium flux in a dose-dependent manner. One of the distinguishing features between the focal adhesion kinases, FAK and PYK2, is the ability to activate PYK2 with calcium. Finally, HGF treatment resulted in higher migrating forms of Paxillin, coinciding with phosphorylation at tyrosine 31 (the first CRKL binding site) but not tyrosines 118 or 181. Paxillin is a dynamic cytoskeletal protein that can form complexes with adhesion proteins, including FAK, PYK2, Src, Csk, and p130Cas (26). Moreover, differential phosphorylation of Paxillin appears to influence the temporospatial regulation of focal adhesions and the actin cytoskeleton (27).

The process of cell motility, as reflected by formation of membrane ruffles and filopodia, as well as migration, is well regulated by small GTPases and PI3k. The actin cytoskeleton needs to be dynamic for cell shape change, alterations in cell contact, and cell motility. Ras-like GTPases of the Rho family, which includes Rho, Rac, and Cdc42, can reorganize actin. In Swiss 3T3 cells, Rho is involved in formation of actin stress fibers and focal adhesions, Rac is involved in formation of lamellipodia and membrane ruffles, and Cdc42 is involved in formation of filopodia (28, 29). In the cascade of regulation, Cdc42 activation is followed by Rac activation, thereafter stimulating Rho. Specific inhibitors of Rho activity, such as C3 ADP-ribosyltransferase and Rho-guanine nucleotide dissociation inhibitor, interfere with Swiss 3T3 cell motility, and this can be reversed by the addition of constitutively Rho. The Rho family members are clearly under tight regulation in the cell, but the mechanisms of regulation are not understood in SCLC and would be quite interesting to study further. We would hypothesize that Rac is important in the membrane ruffling (blebbing) and Cdc42 is important in filopodia formation in SCLC stimulated by HGF. It is also not understood if small GTPases or PI3k affect scattering. However, in the SCLC lines studied, we were not able to appreciate cell scattering (data not shown). This may be because SCLC are suspension cells and may behave differently than epithelial cells.

As another biological function of HGF, we have shown that ROS levels are up-regulated in SCLC. ROS, such as O2 \(^{-}\), OH \(^{-}\), NO \(^{-}\), and H2O2, have recently begun to be appreciated for their role in regulation of signal transduction, gene expression, proliferation, and motility (30, 31). SCLC is directly caused by cigarette smoking, and it is well known that cigarette smoke can generate considerable toxic ROS (32). A delicate balance between oxidants and the protective effects of intra- and extracellular oxidant defense system is abrogated in SCLC. In a recent study from Arakaki et al. (33), it was shown that the N-acetylcysteine prevented HGF-suppressed growth of Sarcoma 180 and Meth A cells and HGF-induced apoptosis. In contrast to the study by Arakaki et al. (33), we did not observe altered apoptosis on HGF stimulation (data not shown) and have found that ROS are generated by HGF, maximally within 60 min of treatment. ROS have been shown to contribute to cellular functions, and we have recently demonstrated that ROS can induce cell cycle progression, increase cell migration, inhibit protein tyrosine phosphatases, and lead to increase phosphorylation of cellular proteins (17). It would now be interesting to study the expression and activity of ROS-regulating enzymes by HGF in SCLC.

HGF also leads to plasmin activation, and Webb et al. (14) have reported that geldanamycins lead to decreased plasmin activation at femtomolar concentrations. Using the geldanamycins at nm concentrations led to down-regulation of e-Met, inhibition of HGF-mediated cell motility and invasion, and reversion of Met-transformed cells. Using geldanamycin in SCLC cells responsive to HGF, we have further shown alteration in cell cycle, apoptosis, decreased cell motility, and decreased tyrosine phosphorylation. Within 72 h of treatment with geldanamycin, there was decreased viability and apoptosis, of all four SCLC cell lines tested.

Geldanamycin has been described as an inhibitor of Hsp90 function (19). Hsp90 functions as a chaperone, to assist proteins to acquire mature conformation, and has been shown to associate with tyrosine kinases, such as v-Src, LCK, and p210BCR/ABL. In a recent study by An et al. (34), geldanamycin altered the association of Hsp90 with p210BCR/ABL, thereby leading to the degradation of the cellular tyrosine kinase. We show in this study that there was indeed decreased amounts of c-Met in response to geldanamycin treatment. However, this may not be related to association with Hsp90, because cross-immunoprecipitation did not reveal any direct interaction between these two molecules. Possibilities are that c-Met either may not be synthesized as fast as nongeldanamycin-treated cells or that c-Met is being degraded via ubiquitination in response to geldanamycin. The striking observation that Hsp70 levels are increased by geldanamycin is unique and has not been reported previously. Hsp70 has been implicated to be important in apoptosis events as related to ROS (35), and the levels of Hsp70 may be increasing with geldanamycin to the oxidative stress response of the cells undergoing apoptosis and needs to be further explored. It is to be noted, although geldanamycin may reduce the expression of c-Met, geldanamycin may also act through other mechanisms than c-Met inhibition alone. This possibility is raised because HGF did not restore the biological functions in geldanamycin-treated cells. It would now be interesting to determine which other pathways, including Hsp90 and Hsp70, would be involved in the biochemical and biological effects of geldanamycin in SCLC. Additionally, there are analogues of geldanamycin available, and the effects on SCLC along with HGF/c-Met should be further explored. Finally, inhibitor specifically targeting HGF/c-Met would be quite useful for SCLC studies.

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