Geranylgeranyl transferase 1 modulates autophagy and apoptosis in human airway smooth muscle

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Ghavami S, Mutawe MM, Schaafsma D, Yeganeh B, Unruh H, Klonisch T, Halayko AJ. Geranylgeranyl transferase 1 modulates autophagy and apoptosis in human airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 302: L420–L428, 2012. First published December 9, 2011; doi:10.1152/ajplung.00312.2011.—Geranylgeranyl transferase 1 (GGT1) is involved in the posttranslational prenylation of signaling proteins, such as small GTPases. We have shown that blocking the formation of isoprenoids with statins regulates survival of human lung mesenchymal cells; thus, we tested the hypothesis that GGT1 may specifically modulate programmed cell death pathways in these cells. To this end, human airway smooth muscle (HASM) cells were treated with the selective GGT1 inhibitor GGTi-298. Apoptosis was seen using assays for cellular DNA content and caspase activation. Induction of autophagy was observed using transmission electron microscopy, immunoblotting for LC3 lipidation and Atg5-12 complex content, and confocal microscopy to detect formation of lysosome-localized LC3 puncta. Notably, GGT1 inhibition induced expression of p53-dependent proteins, p53 upregulated modulator of apoptosis (Noxa), and damage-regulated autophagy modulator (DRAM), this was inhibited by the p53 transcriptional activation inhibitor GGTi-298. Overall, we demonstrate for the first time that pharmacological inhibition of GGT1 induces simultaneous p53-dependent apoptosis and autophagy in HASM. Moreover, autophagy regulates apoptosis induction. Thus, our findings identify GGT1 as a key regulator of HASM cell viability.

GGTi-298; p53; Rho; GTPase; cell death

AIRWAY DISEASES, INCLUDING asthma and chronic obstructive pulmonary disease, are characterized by structural changes of the airway wall (airway remodeling) (37, 41, 60). Airway remodeling has a fibroproliferative origin, which includes accumulation of airway smooth muscle (ASM), likely through altered myocyte growth and survival (1, 32). Increased ASM mass is considered the major causal factor of dyspnea in asthma due to its impact on airway narrowing (3, 6, 11). Indeed, ablation of ASM mass by means of bronchial thermoplasty in dogs reduced airway responsiveness by ~50% for up to three years (18). Furthermore, in human asthmatic subjects, thermoplasty-mediated ASM removal improved disease symptoms, including reduced bronchial hyperresponsiveness persisting beyond 12 months (14). These observations highlight the potential effectiveness of interventions that can lead to reduced ASM mass in asthmatic subjects. To date, no pharmacological approach achieves this endpoint.

The isoprenoids, farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP), are principal substrates for heterodimeric farnesyl transferase (FT) and geranylgeranyl transferase (GGT) 1, respectively. These transferases catalyze lipid modification of small GTPases (monomeric G proteins), for instance, FT selectively prenylates H-Ras whereas GGT1 catalyzes lipid modification of Rho, K-Ras, Rap, and Rab. Posttranslational isoprenylation underpins GTPase membrane anchoring, activation, and association with downstream effectors (55–56). This is a determinant of cell survival (62), proliferation (61), adhesion (46), extracellular matrix protein synthesis (47), and malignant transformation (36, 48, 59, 65).

Inhibition of protein geranylgeranylation induces cell death in a broad range of cells from different origins (10, 21, 49). Moreover, GGT1 has emerged as an upstream regulator of smooth muscle cell responses that include cell survival, proliferation, extracellular matrix expression and deposition, contraction, cyclooxygenase expression, and generation of reactive oxygen species (20, 53, 61, 63). We recently showed that depletion of cellular pools of FPP and GGPP by blocking the cholesterol/mevalonate metabolic pathway prevents RhoA activation and induces p53-dependent apoptosis and autophagy in human airway mesenchymal cells (27, 29). Interestingly, depletion of FPP and GGPP to promote autophagy countered mesenchymal cell apoptosis, likely through turnover of dysfunctional mitochondria that would otherwise release damaging reactive oxygen species and cytochrome c. However, autophagy, or programmed “self eating,” is also widely recognized as a mechanism to ensure the availability of ATP stores to support energy-requiring processes, including apoptosis; thus, autophagy can play a context-specific dual role in relation to cell survival and growth (2). Despite a role for geranylgeranylation of central signaling proteins in cell survival and growth, a precise role for GGT1 in regulating apoptosis, autophagy, and cell fate has not been elucidated.

In the current study, we tested the hypothesis that GGT1 modulates human airway smooth muscle (HASM) cell apoptosis and autophagy via pathways that exert coordinated control of these processes. To this end, using primary cultures of HASM cells, we tested the effects of the GGT1 selective inhibitor GGTi-298 (8, 37, 68) on both apoptosis and autophagy and determined the signaling pathways that regulate these responses. Our findings allow us to more precisely pinpoint how GGT1 regulates cell survival pathways and specifically implicate p53 signaling as a principal downstream target.

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MATERIALS AND METHODS

**Materials.** Cell culture plasticware was obtained from Corning Costar. Cell culture media, propidium iodide (PI), GGTTi-298, cyclic pifithrin-α, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), rabbit-anti LC3, and bafloymycin A1 (Baf-A1) were obtained from Sigma-Aldrich (Oakville, CA). Rabbit anti-human cleaved caspase-6, -7, and -9; rabbit anti-human proteins p53 upregulated modulator of apoptosis (PUMA), Bax, and Atg5-12; and mouse anti-RhoA, Cdc42, and Rac1/2/3 were purchased from Cell Signaling. Rabbit anti-damage-regulated autophagy modulator (DRAM), mouse anti-p53, and scrambled and Atg7 short-hairpin RNA (shRNA) were purchased from Santa Cruz Biotechnologies. Rabbit anti-p53, and scrambled and Atg7 short-hairpin RNA (shRNA) were purchased from R&D.

**Primary HASM cell culture preparation.** For all experiments, primary HASM cells were isolated from macroscopically healthy segments of second- to fourth-generation main bronchi obtained after resection surgery from patients with adenocarcinoma. After microdissection to separate the lamina reticularis and submucosal compartment from encircling ASM bundle, HASM cells were isolated by enzymatic dissociation as we have described (60). All procedures were approved by the Human Research Ethics Board (University of Manitoba, ethic reference number:H2002:150). For all experiments, HASM cells from passages 2–5 were used. Cells were grown to ~80% confluence in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 50 U/ml streptomycin, 50 µg/ml penicillin, and 10% FBS. For all experiments, cells were starved in DMEM containing 0.5% FBS for 48 h before all treatments, which were also carried out in DMEM + 0.5% FBS. Medium was changed every 48 h.

**Quantitative RT-PCR for GGTT subunits.** Total cellular RNA was isolated using the RNeasy Plus Mini Kit (Qiagen, Mississauga, ON) and then one reverse transcription using the Quantitec Reverse Transcription Kit. The abundance of farnesyltransferase, CAAX box, α (FNTA) and GGTTβ mRNA was determined using the Applied Biosystems 7500 Thermocycler and the Power SYBR Green PCR Master Mix. Oligonucleotide primers used and product sizes are summarized in Table 1. The PCR conditions were as follows: 94°C for 2 min and then 33 cycles (94°C for 45 s, 60°C for 1 min, and 72°C for 1 min) and for final extension of 72°C for 5 min. A dissociation curve was generated at the end of each PCR reaction to verify that a single product was amplified. 18S rRNA and primers 5′-CGCCTACTAGG-GTGAATCTC-3′ (forward) and 5′-TTGGCAATAGCTTTCCGTCT-3′ (reverse) served as the endogenous reference gene. The relative expression levels of FNTA and GGTTβ were normalized to 18S rRNA (27, 29).

**Cell viability assay.** HASM cell viability was determined under various treatment conditions using MTT as we have described previously (23, 24).

**Measurement of apoptosis by flow cytometry.** Apoptosis was measured using the Nicoletti method (25, 51). Briefly, cells were treated with GGTTi-298 and then harvested and resuspended (30 min, 30°C) in hypotonic PI lysis buffer (1% sodium citrate, 0.1% Triton X-100, 0.5 mg/ml RNase A, and 40 µg/ml PI) and subsequently analyzed by flow cytometry. Nuclei to the left of the G1 peak containing hypodiploid DNA were considered apoptotic.

**Membrane anchoring of rho GTPases.** For determination of membrane anchoring of prenylated Rho and Rac GTPases, HASM cells were kept in DMEM/0.5% FBS in the presence or absence of GGTTi-298 (20 µM) and then scraped in ice-cold buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, and protease inhibitor cocktail), sonicated, and subjected to ultracentrifugation (100,000 g for 35 min) to separate cytoplasmic and membrane fractions (27). The membrane fractions were solubilized in dissociation buffer (50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 1 mM dithiothreitol, 1% SDS, 1 mM EDTA, 1 mM EGTA, and protease inhibitor cocktail) and size fractioned by SDS-PAGE for immunoblot analysis using anti-Rac1/2/3 and anti-RhoA primary antibodies.

**Luminescence caspase activity assays.** Caspase-Glo-9, -3/7, and -8 (Promega) were used to measure the proteolytic activity of caspase-3/7 (DEVD-ase), -9 (LEHD-ase), and -8 (Z-LETD-ase) as previously described (27).

**Immunoblotting.** Protein lysates were prepared in lysis buffer [20 mM Tris-HCl (pH 7.5), 0.5% Nonidet P-40, 0.5 mM phenylmethyl-sulfonyl fluoride, 100 µM β-glycerol 3-phosphate, and 0.5% protease inhibitor cocktail]. Protein content in supernatants was determined using the Lowry Protein Assay (Bio-Rad). Samples were fractionated by SDS-PAGE and transferred to nitrocellulose membranes under reducing conditions. After membranes were blocked with nonfat powdered milk and Tween 20, blots were incubated overnight with the primary antibodies at 4°C. Primary antibody dilutions used were: 1:1,000 for anti-caspase-3, -6, -7, and -9, poly(ADP-ribose)polymerase (PARP), PUMA, RhoA, Cdc42, Rac1/2/3, Atg5-12, and Bax; 1:750 for anti-NOXA; 1:500 for p53; 1:2,500 for LC3-β; and 1:3,000 for GAPDH. Horseradish peroxidase-conjugated secondary antimouse Ig was used at 1:3,000 and anti-rabbit Ig at 1:5,000. Secondary antibody incubation was for 1 h at room temperature, and then blots were developed by enhanced chemiluminescence detection (Amer sham-Pharmacia Biotech) (25).

**Immunocytochemistry, confocal imaging, and electron microscopy.** For immunocytochemistry, HASM cells were grown overnight on cover slips and then treated with GGTTi-298 (20 µM) or vehicle for 72 h before fixation with 4% paraformaldehyde/120 mM sucrose (15 min, 4°C) and permeabilization with 0.1% Triton X-100/4% paraformaldehyde (5 min, 4°C). Cells were incubated with rabbit anti-LC3-β (1:250). Fluorescent images were captured and analyzed using an Olympus Fluoview multilaser confocal microscope. Where needed, lysosomes were stained with 250 nM Lysotracker Red (Invitrogen Molecular Probes) before fixation (27).

For transmission electron microscopy (TEM), cells were fixed (2.5% glutaraldehyde in PBS, pH 7.4) for 1 h at 4°C and then postfixed in 1% osmium tetroxide before embedding in Epon. TEM was performed with a Philips CM10, at 80 kV, on ultrathin sections (100 nm on 200 mesh grids) stained with uranyl acetate and counterstained with lead citrate (25).

Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Product Size, bases</th>
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<tbody>
<tr>
<td>FNTA</td>
<td>Forward</td>
<td>TATAGATCCCGGTGCCCGAGATA 95</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>ACTTCTCGGGAAAAATGCCACCTGT 142</td>
</tr>
<tr>
<td>PGGTβ</td>
<td>Forward</td>
<td>TTGAATGGACTCTAGTGGCTCT 137</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>TCAGTGCTCTAGTGGCTGACA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward</td>
<td>AGGAATGGCTCTGGCACGCAAC</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AGACTGGAGATGGCGCCCTCGG</td>
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FNTA, farnesyltransferase, CAAX box, α; PGGTβ, protein geranylgeranyl transferase type 1 beta subunit; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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Stable gene silencing: lentiviral delivery of shRNA. Atg7 shRNA- and noncoding shRNA-lentiviral particles (Santa Cruz) were used to directly infect immortalized HASM cells, and stable clones were selected using puromycin (29). Human bronchial smooth muscle cell lines immortalized by stable expression of human telomerase reverse transcriptase were prepared as described previously (60).

Statistical analysis. All results were expressed as means ± SD and were compared by one-way or two-way ANOVA followed by Tukey’s or Bonferroni’s post hoc test, using Graph Pad Prism 5.0. P < 0.05 was considered significant. Data were collected in triplicate from at least three cell lines unless otherwise indicated.

RESULTS

Inhibition of GGT1 induces apoptosis in HASM cells. Consistent with our previous findings (58), subunits required for the formation of GGT1 and FT were expressed in all four HASM cell lines used for our studies (Fig. 1A). We found that...
direct inhibition of GGT1 with GGTi-298 induced cell death in HASM cells (Fig. 1B). We further confirmed that GGTi-298 induced apoptosis using both FACS analysis of nuclei stained with PI (20 \( \mu M \), \( P < 0.01 \), Fig. 1, C and D) and with phase-contrast microscopy that revealed GGTi-298-induced cellular shrinkage and condensation (Fig. 1E). Because the effects of GGT1 inhibition may be mediated through downstream protein targets that include monomeric GTPases (27, 52, 58), we assayed the impact of GGTi-298 on basal RhoA, Cdc42, and Rac1/2/3 membrane localization and found that these became markedly reduced in HASM cells (Fig. 1F).

**GGTi-298 induces intrinsic apoptosis pathways in HASM.** GGTi-298 (20 \( \mu M \)) induced transient cleavage of the intrinsic pathway effector caspase-9, as well as execution caspase-3, -7, and -6 (Fig. 2A). This effect reached a peak 96 h after initiating treatment. In contrast, extrinsic pathway-associated caspase-8 was not activated by GGT1 inhibition (Fig. 2B).

**GGT1 inhibition stimulates p53-dependent apoptosis and autophagy in HASM.** Because the proapoptotic function of p53 occurs both at the level of transcriptional activation of genes for proapoptotic proteins such as Puma, Noxa, and Bax and through interaction in the cell cytosol with proteins of the Bcl-2 family of apoptosis regulators (27, 29, 44), we next measured the impact of GGTi-298 on the abundance of p53 protein and found it accumulated significantly (Fig. 3A). Furthermore, the elevation in p53 correlated with a subsequent increase in PUMA, Noxa, and Bax in HASM cells.

Numerous reports indicate the coexistence of autophagy and apoptosis signaling pathways under many conditions, and p53 has been identified as a potential coregulator (16, 29, 33, 38, 40, 42). Thus, we next investigated if inhibition of GGT1 also promoted autophagy in HASM cells; indeed, GGTi-298 (20 \( \mu M \)) induced features of autophagy, including LC3 lipidation, LC3-II formation, and increased levels of DRAM, a specific marker for p53-induced autophagy (Fig. 3B). To further confirm the induction of cellular autophagy, we also performed TEM, which revealed the formation of double-membrane autophagosomes, autophalysosomes, and the occurrence of autophagosome and lysosome fusion (Fig. 3C). The latter was also confirmed by confocal microscopy, which revealed the colocalization of LC3 and activated lysosomes (Fig. 3D).

In a final set of experiments, we observed that induction of p53-dependent apoptosis proteins (PUMA, Noxa, and Bax) and concomitant cell death were both prevented using the p53-specific transcriptional inhibitor cyclic pifithrin-\( \alpha \) (5 \( \mu M \)) (Fig. 4, A and B). Furthermore, cyclic-pifithrin-\( \alpha \) also inhibited GGTi-298-induced LC3 lipidation and DRAM expression. Collectively these findings confirm an effector role for p53 in both GGTi-298-induced apoptosis and autophagy.

**Autophagy counterbalances GGT1 inhibition-induced apoptosis.** Several studies reveal that apoptosis and autophagy can be integrated and regulated by similar cues (25, 29, 31). Thus, we investigated interplay between GGT1 inhibition-induced autophagy and apoptosis in HASM cells. Baf-A1 (0.01 \( \mu M \)), which inhibits late autophagy events, augmented both GGTi-298-induced cell death (\( P < 0.001 \), Fig. 5A) and the cleavage of caspase-7 and PARP (Fig. 5B). The mechanism of action of Baf-A1 is to reduce autophagy flux through blockade of autophagosome-lysosome fusion; thus, LC3\( \beta \)-II-rich vesicles accumulate, an effect confirmed by the increased abundance of LC3\( \beta \)-II in cells cotreated with Baf-A1 and GGTi-298 (Fig. 5B). Using shRNA to silence Atg7, a protein that promotes LC3 lipidation during autophagy (50), we observed enhanced GGTi-298-induced cell death (\( P < 0.001 \), Fig. 5C), caspase-7 and PARP cleavage, and, as predicted, decreased LC3 lipidation (Fig. 5D). Collectively these data indicate that GGT1 inhibition permits induction of autophagy, and this effect negatively modulates proapoptotic effects of GGTi-298 that would otherwise lead to rapid cell death.

**DISCUSSION**

We have recently shown that inhibiting proximal steps of the mevalonate cascade with a statin (simvastatin) induces p53-dependent autophagy and apoptosis in primary human lung mesenchymal cells grown in media containing low levels of mitogen (27, 29). In the present study, we extend these findings using the specific inhibitor GGTi-298 to elucidate a role for a

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**Fig. 2.** GGTi-298 induces intrinsic caspase-dependent apoptosis in primary HASM cells. A: immunoblot detection of cleaved caspase-3, -6, -7, and -9 showing time-dependent caspase activation with GGTi-298 (20 \( \mu M \)) treatment. For all lanes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a protein loading control. Blots are typical of two replicates using three different primary HASM cultures. B: effects of GGTi-298 treatment (20 \( \mu M \), 72 h) on caspase-8, caspase-3/-7, and caspase-9 enzymatic activity, as detected by Caspase-Glo luminometric assay. Caspase activity is presented as normalized to that measured for solvent-only treated cultures. The data represent means ± SD of duplicate experiments performed on 3 different primary HASM cell lines. NS, not significant. ***\( P < 0.001 \) compared with untreated controls.
We demonstrate that GGT1 comodulates autophagy and apoptosis, and this is linked to mechanisms involving p53. Autophagy has emerged as a negative regulator of simvastatin-induced apoptosis in prior work with human lung cells (29). Our present study demonstrates that GGT1 is likely a key coordinator of this effect. This is in line with evidence from cancer cells in which pharmacological inhibition of GGT1 or depletion of GGPP can provoke apoptosis (7, 15, 49) and/or autophagy (57, 67). To our knowledge, our study is the first to report a role for GGT1 in autophagy and apoptosis in airway mesenchymal cells. Moreover, we reveal that GGT1 is a determinant of ASM cell survival, suggesting that it should be considered for its possible contributions to wound healing and remodeling in the lungs during development and the pathogenesis of diseases such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease.

It has been known for some time that the oncogene p53 has proapoptotic activity (27, 44). The apoptotic function of this protein is mediated both through transcriptional activation of proapoptotic proteins such as PUMA, NOXA, and Bax and through its ability to bind to (and inhibit) antiapoptotic proteins such as Bcl-XL (9, 27, 43). More recently, p53 has also been found to be involved in the control of autophagy through the induction of DRAM, a transmembrane endoplasmic reticulum protein that compromises lysosomal function to modulate au-

Fig. 3. GGTi-298 induces p53-dependent apoptosis and autophagy in primary HASM. A: immunoblot showing the accumulation of cell death-related proteins that are transcriptionally regulated by p53. HASM cells were treated with GGTi-298 (20 μM) for up to 120 h as indicated. Immunoblot was performed using total cell extracts resolved by SDS-PAGE. GAPDH was used as a loading control for all experiments. Shown data are typical of 3 independent experiments. B: p53-dependent autophagy induction confirmed by accumulation of p53, damage-regulated autophagy modulator (DRAM), LC3β lipidation (LC3β II), and Atg5-12 conjugation in HASM cells treated with GGTi-298 (20 μM) for up to 120 h. Immunoblot was performed using total cell extracts resolved by SDS-PAGE. GAPDH was used as loading control for all experiments. Shown data are typical of 3 independent experiments. C: HASM cells were treated with 20 μM GGTi-298 for 72 h (row on top, panel on right) and then imaged by transmission electron microscopy (TEM) (magnification: 4.6 × 103). An untreated, control cell is shown in the top left panel for reference. Both autophagolysosomes (indicated by arrow) and autophagosome-lysosome fusion events (indicated by arrowhead) were evident. The panels on the bottom row are enlarged regions from the top right image showing: typical autophagolysosome (arrow) (left) and an autophagosome-lysosome fusion event (arrowhead) (bottom right). a, Autophagolysosome; L, lysosome; n, nucleus. D: HASM cells treated with GGTi-298 (20 μM, 72 h) showed increased Lysotracker Red staining (a marker of lysosomal activation), an increase in punctuate staining for LC3β (green), a marker of autophagy, and LC3β lysosomal colocalization.
tophagy-initiating proteins (16, 22, 29, 38). The involvement of p53 in GGTI-induced apoptosis in cancer cells has also been reported (45), but there are conflicting reports (17, 66). In our study, we found that GGT1 activity was required to prevent the activation of both p53-dependent autophagy (e.g., increased DRAM) and apoptosis (e.g., increased PUMA, NOXA, and Bax). Moreover, we show evidence for GGT1 control of other hallmarks of cellular apoptosis (e.g., caspase activation and cell shrinkage) and autophagy (e.g., LC3 lipidation, autophagosome formation, and lysosomal activation and colocalization with LC3). We demonstrated the involvement of p53 transcriptional activity in GGTI-298-induced apoptosis and autophagy responses, since these could be prevented by the p53 transcriptional inhibitor cyclic-pifithrin-α. To our knowledge, these observations are among the first to identify GGT1 as a regulator of p53 and its role in survival of somatic lung cells.

Protein prenylation and membrane translocation of monomeric GTPases play important roles in cell function, including

Fig. 4. Transcriptional inhibition of p53 decreases GGTI-298-induced cell death and reduces p53-dependent apoptosis and autophagy markers. A: cyclic pifithrin-α decreases GGTI-298-induced cell death. Where indicated, HASM cells were pretreated with cyclic-pifithrin-α (10 μM, 4 h) before GGTI-298 (20 μM) cotreatment for an additional 96 h. Cell viability was assessed using MTT assay. Pifithrin-α significantly decreased GGTI-298-induced cell death in HASM (***P < 0.001). Data shown are the means of 6 experiments performed in quadruplicate. B: HASM cells were pretreated with cyclic-pifithrin-α (4 h, 10 μM) before GGTI-298 exposure (20 μM) for 48 and 96 h. Cyclic pifithrin-α decreased p53 upregulated modulator of apoptosis (PUMA) and DRAM expression and also LC3β lipidation (LC3β II). Immunoblotting was performed using total cell extracts resolved by SDS-PAGE. GAPDH was used as a loading control for all experiments. Shown data are typical of 3 independent experiments.

Fig. 5. Autophagy inhibition augments GGTI-298-induced apoptotic cell death in HASM. A: HASM cells were pretreated with Baf-A1 (0.01 μM, 4 h) and then cotreated with GGTI-298 (20 μM, 96 h). MTT assay showed a significant increase of GGTI-298-induced cell death in HASM after treatment with Baf-A1 (***P < 0.001). Data represent means ± SD of 3 experiments completed in triplicate in 3 primary cell lines. B: Baf-A1 and GGTI-298 cotreatment also increased hallmarks of apoptosis (cleaved caspase-7 and cleaved PARP) and accumulation of LC3β II in HASM. Immunoblotting was performed using total cell extracts resolved by SDS-PAGE. GAPDH was used as a loading control for all experiments. Shown data are typical of 3 independent experiments. C: cell viability assay (MTT assay) using control and ATG7 shRNAi HASM cells, with and without GGTI-298 treatment (20 μM, 96 h), showed that ATG7 shRNAi significantly enhanced GGTI-298-induced cell death (P < 0.001). Data represent means ± SD of experiments completed in triplicate in 2 primary cell lines. D: immunoblot showing effects of GGTI-298-treated HASM after infection with lentivirus harboring control or ATG7 shRNAi. Specific antibodies against the indicated proteins were used, with GAPDH serving as a loading control. Atg7 silencing decreases LC3β lipidation while it increases caspase-7 and PARP cleavage. Shown data are typical of 2 independent experiments.
cell growth and survival (4–5, 13, 27, 29). This mechanism involves the addition of C20 (geranylgeranyl) to the COOH-terminus of the G protein by GGT1 (68). The inhibition of protein geranylgeranylation and its link to activity of small GTPases has received attention for potential as a cancer therapy, in particular those involving mutations of Ras (7, 15, 49, 64). Here we show that GGTi-298 attenuates membrane anchoring and promotes relocalization of RhoA, Cdc42, and Rac1/2/3 to the cytosol where activity is known to be reduced. There are several reports about the role of Rho family members in apoptosis, autophagy, and cell death in cancer cells (54), for example, human thyroid carcinoma, but also in somatic cells such as cardiac (myo)fibroblasts (12, 69) and osteoclasts (35). These reports and our current findings are consistent with recent reports of small GTPase regulation of p53-mediated cell migration (34), senescence (19), and apoptosis (39); thus, our data increase understanding of how these signaling proteins could be targeted to regulate ASM growth in asthma.

Numerous recent review and research articles indicate a correlation between autophagy and apoptosis signaling pathways (29, 33, 40, 42). Currently, the determinants for whether autophagy contributes to cell death after cytotoxic stimulation or for it to act as a protector against cell death are not fully understood. Under inauspicious metabolic conditions, such as nutrient deficiency or hypoxia, autophagy is enhanced, leading to delipidation of proteins and organelles to supply amino acids, fatty acids, and nucleotides such as ATP for essential cell processes. Indeed, because apoptosis is an ATP-requiring process, autophagy can serve to promote cell death (30). However, in our study, we show that GGTi-298-mediated autophagy protects against apoptosis, as evidenced by increased cell death and apoptotic marker expression when proautophagy Atg7 is silenced. These findings correlate with our previous observations showing autophagy is a negative regulator of mevalonate cascade inhibition-induced apoptosis (29). Furthermore, they are consistent with a growing body of evidence showing autophagy prevents apoptotic cell death (29). Clearly, the balance of autophagy and apoptosis is highly regulated by multiple mechanisms under different conditions. Our study suggests GGT1 may be among the determinants of lung cell responses to factors that impact cell growth and survival in normal and pathophysiological conditions.

In conclusion, our study shows that basal GGT1 activity suppresses HASM cell autophagy and apoptosis, likely via mechanisms that prevent induction of p53 signaling. Thus, selective pharmacological inhibition of GGT1 induces a novel cell death mechanism in HASM cells that involves p53-dependent autophagy and apoptosis. In this process, autophagy regulates the extent to which apoptosis occurs. In this context, our findings identify GGT1 as a regulator of HASM cell viability; however, future studies are still required to determine whether the pathways and signaling proteins identified in our current study are important determinants of cell responses in pathophysiological conditions. Nonetheless, our findings do suggest that, in a manner similar to that of cancer research circles, the pharmacological targeting protein geranylgeranylation may offer potential for therapies to be developed for fibroproliferative lung diseases that involve mesenchymal cells.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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inhibiting the RhoA/ROCK pathway and reducing MMP-9 mRNA levels. 


