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FOLLICULAR THYROID CANCER CELL GROWTH INHIBITION BY PROTEOSOME INHIBITOR MG132

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Abstract

Background—Effective therapies for the subset of follicular thyroid cancer (FTC) patients with aggressive, metastatic disease are lacking. Therefore, we sought to determine the effects of proteasome inhibition, an emerging class of chemotherapeutic agents, on metastatic FTC cells.

Materials and Methods—Human metastatic FTC cells (FTC236) were treated *in vitro* with the proteasome inhibitor MG132 (0 to 800 nM). Western blot analysis was performed on whole cell lysates isolated after 2 days. To measure cell growth, we performed an MTT cellular proliferation assay over 6 days.

Results—Treatment of FTC236 cells with MG132 led to dose-dependent cell growth inhibition. Increases in inactive, phosphorylated GSK-3 β and active β -catenin also were observed. With 800 nM MG132, growth was reduced by 87% at 6 days ($P < 0.0001$). This reduction in cellular proliferation correlated with the degree of GSK-3 β inhibition. MG132 treatment also caused increased p21^{Waf1/Cip1} and decreased cyclin D1 expression suggesting that growth suppression may occur through cell cycle arrest.

Conclusion—Growth of metastatic human FTC cells appears to be suppressed by proteasome inhibition. Whether this effect is directly due to cell cycle arrest and inactivation of GSK-3 β signaling is unclear. Nonetheless, these compounds may become novel treatments for aggressive, metastatic FTC.

Keywords

MG132; proteasome inhibitor; follicular thyroid cancer; glycogen synthase kinase-3 β ; β -catenin; FTC236

INTRODUCTION

Follicular thyroid cancer (FTC) accounts for approximately 10–15% of all thyroid cancers and is second only to papillary thyroid cancer (PTC) in incidence (1). While FTC does occur in people of all ages, the majority of patients are female and between 30 to 50 years old (1,2). Thyroid cancer management has been fairly consistent over the last decade consisting of total

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or near-total thyroidectomy, thyroid remnant ablation with I^{131} radioactive iodine therapy, and suppression of TSH. Most patients who receive this therapy achieve a disease-free outcome (3,4). Compared to PTC, FTC is often diagnosed at a later stage with a more aggressive tumor and a moderately worse prognosis (4). Patients with FTC also are at a greater risk for the development of distant metastases due to an increased tendency to invade the vasculature (2). In addition, patients with aggressive, metastatic FTC may be unresponsive to radioiodine treatment because the tumor cells lose their ability to concentrate radioiodine over time. The inability to respond to radioactive iodine therapy is most likely due to loss of the human sodium-iodide symporter (hNIS) function (4,5). As a consequence, large, aggressive FTCs are not responsive to the conventional therapies. Therefore, alternatives strategies are needed for treating patients with aggressive, metastatic FTC.

The growth of cancers such as FTC is regulated via a complex network of signal transduction pathways. The glycogen synthase kinase-3 β (GSK-3 β) signaling pathway has been identified as a potential target for novel cancer therapies (6). GSK-3 β is an important regulator of several cellular processes including development, metabolism, gene transcription, cell cycle progression, proliferation, and apoptosis through its action as a serine/threonine protein kinase (6,7). Inactivation of GSK-3 β is thought to contribute to inhibition of tumor cell growth. Expression levels of the active, unphosphorylated form of GSK-3 β have been shown to be higher in tumor cells than in their normal counterparts (7). Active GSK-3 β is considered to promote tumor growth, whereas inactivate, phosphorylated GSK-3 β inhibits cellular growth. GSK-3 β also is responsible for constitutive phosphorylation of β -catenin which is necessary for the ubiquitin-proteasome mediated degradation of β -catenin protein. In other tumor models, inhibition of GSK-3 β by phosphorylation of a single serine residue (Ser⁹) not only inhibits tumor growth, but also contributes to an accumulation of β -catenin, a key component of the Wnt signaling pathway (6,8,9). When increased levels of β -catenin form, this molecule translocates into the nucleus and interacts with various transcription factors to regulate gene expression (8,9).

The ubiquitin-proteasome system (UPS) recently has been the focus of several studies involved in the development of new cancer therapies (10–12). The 26S proteasome is a large, multicatalytic threonine protease that provides the major pathway for degradation of ubiquitinated intracellular proteins (10). This 26S proteasome is involved in the targeted degradation of key regulatory proteins necessary for cell-cycle progression and apoptosis in normal and malignant cells. Therefore, the 26S proteasome has become a potentially important therapeutic target in diseases of cell proliferation, including cancer (11,12). Many findings conclude that actively proliferating malignant cells are more sensitive to proteasome inhibition than non-cancerous cells. Consequently, novel cancer therapies are exploring the use of proteasome inhibitors against several kinds of tumors (12). MG132 is an example of one of the first described synthetic peptide aldehyde proteasome inhibitors that is both potent and reversible (11).

In this study, we investigated the effects of the proteasome inhibitor MG132 on a human, metastatic FTC cell line, FTC236. An increase in inactive, phosphorylated GSK-3 β was observed in FTC236 cells treated with MG132. β -catenin, a downstream molecule of GSK-3 β , also increased in expression. FTC cell growth was significantly reduced by MG132 treatment in a manner that was proportional to the degree of GSK-3 β phosphorylation. In addition, MG132 treatment led to a decrease in p21^{Waf1/Cip1} and an increase in cyclin D1 suggesting that cellular proliferation was inhibited through cell cycle arrest. As a result, proteasome inhibition may be an acceptable novel therapeutic approach for treating aggressive, metastatic FTC.

MATERIALS AND METHODS

Cell Culture

FTC236 cells (a human metastatic FTC cell line isolated from a lymph node) provided by Dr. Fiemu Nwariaku (UT Southwestern, Dallas, TX) were maintained in Dulbecco's Modified Eagle/Ham's F-12 (1:1; Invitrogen, Carlsbad, CA) medium supplemented with 10% fetal bovine serum, 0.01 IU/mL thyroid-stimulating hormone, 40 µg/mL insulin (Sigma-Aldrich, St. Louis, MO), and 50 µg/mL penicillin/streptomycin (Invitrogen).

Cell Proliferation Assay

Cellular growth was measured by the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) rapid colorimetric assay (Sigma-Aldrich) as previously described (13). Briefly, cells were seeded in quadruplicate on 24-well plates and incubated for 24 hours under standard conditions to allow cell attachment. FTC236 cells were then treated with varying concentrations of MG132 (0 to 800 nM) and incubated for up to 6 days. The media was changed every 2 days with the appropriate concentration of MG132. The MTT assay was performed by replacing the standard medium with 250 µl of serum-free medium containing 0.5 mg/mL MTT and incubating for 3 hours at 37°C. After incubation, 750 µl of dimethyl sulfoxide (DMSO, Sigma-Aldrich) was added to each well and mixed thoroughly. The plates were then measured at 540 nM using a spectrophotometer (µQuant; Bio-Tek Instruments, Winooski, VT). To confirm results, the experiment was repeated.

Western Blot Analysis

FTC236 cells were treated with varying concentrations of MG132 (0 to 800 nM) for 2 days, and whole-cell lysates were prepared as previously described (14). Total protein concentrations were quantified using a bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL). Denatured cellular extracts (30 µg) were resolved using either 10% or 4–12% NuPAGE® Novex® Bis-Tris Mini Gels (Invitrogen) and transferred onto nitrocellulose membranes (BioRad Laboratories, Hercules, CA). The membranes were then blocked in milk (5% nonfat dry milk and 0.05% Tween 20 in 1x phosphate-buffered saline) and incubated with the appropriate antibodies. Primary antibody dilutions were as follows: 1:500 for β-catenin, 1:1,000 for GSK-3β, phosphorylated GSK-3β (pGSK-3β), and phosphorylated β-catenin (pβ-catenin), 1:2,000 for cyclin D1 and p21^{Waf1/Cip1} (Cell Signaling Technology, Beverly, MA), and 1:10,000 for glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Trevigen, Gaithersburg, MD). Horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies (Pierce Biotechnology) were used depending on the source of the primary antibody. Protein signal visualization was performed using membranes developed by Immunar (BioRad Laboratories) for GSK-3β, pGSK-3β, β-catenin, cyclin D1, and GAPDH or by SuperSignal West Femto chemiluminescence substrate (Pierce Biotechnology) for pβ-catenin and p21^{Waf1/Cip1} per the manufacturer's instructions.

Statistical Analysis

ANOVA was performed using a statistical analysis software package (SPSS version 10.0, SPSS, Chicago, IL). Statistical significance was defined as a *P*-value of < 0.05.

RESULTS

MG132 treatment caused inactivation of GSK-3β in FTC236 cells

MG132 treatment of FTC236 cells resulted in progressive phosphorylation of GSK-3β, suggesting a dose-dependent inhibition of the GSK-3β signaling pathway (Fig 1). Phosphorylation and, thus, inactivation of GSK-3β caused by MG132 treatment was

demonstrated by western blot analysis. Phosphorylation of GSK-3 β was demonstrated at MG132 concentrations as low as 50 nM. Control (untreated) cells had no phosphorylated GSK-3 β , implying that GSK-3 β is in an active form at baseline in FTC236 cells. Our western blot analysis also revealed that MG132 treatment did not significantly change levels of unphosphorylated GSK-3 β (Fig. 1). These results showed that GSK-3 β signaling is inactivated in FTC cells by treatment with the proteasome inhibitor, MG132.

β -catenin levels increased in FTC236 cells treated with MG132

Western blot analysis also was used to demonstrate the accumulation of β -catenin, a downstream component of GSK-3 β signaling, in FTC236 cells treated with MG132. When β -catenin is phosphorylated by active GSK-3 β , this protein undergoes rapid degradation by proteasomes in a ubiquitin-dependent manner. Treatment of FTC cells with increasing concentrations of MG132 (0 to 800 nM) led to a progressive increase in β -catenin expression (Fig. 1). Levels of p β -catenin were relatively constant across all treatment groups (Fig. 1). Our results indicated that MG132 inhibited proteasome degradation causing a build up of β -catenin which is a downstream component of GSK3- β signaling.

MG132 suppressed FTC236 cell growth in vitro

Next we examined the effects of MG132 on FTC cell growth using an MTT cellular proliferation assay to measure cell viability. Treatment of FTC236 cells for up to 6 days with MG132 (0 to 800 nM) resulted in a profound dose-dependent inhibition of cell growth (Fig. 2). At the highest concentration of MG132 (800 nM), FTC cell growth was reduced 92% after 6 days ($P < 0.0001$ vs control). Cell proliferation also was inhibited at 6 days with even the lowest concentration of MG132 (50 nM) used (15%; $P < 0.005$ vs control). After only 2 days of treatment, cell growth was significantly suppressed by 100 nM MG132 (17%; $P < 0.01$). These reductions in cellular proliferation were directly proportional to the degree of GSK-3 β inhibition and β -catenin accumulation. These results established that treatment of human FTC236 cells with the proteasome inhibitor, MG132, leads to a reduction in cellular growth.

Proteasome inhibition induced growth suppression of FTC cells through cell cycle arrest

After determining that cell proliferation was inhibited by MG132 treatment, we wanted to investigate the mechanism of action responsible for this effect. Western blot analysis was performed on lysates from FTC236 cells treated with MG132 for 48 hours for various markers of cell cycle arrest and apoptosis. Treatment of FTC cells with increasing concentrations of MG132 (0 to 800 nM) resulted in an increase in protein levels of the cyclin-dependent kinase inhibitor p21^{Waf1/Cip1} and suppression of the cell cycle promoter cyclin D1 (Fig. 3). These two proteins help control progression through the cell cycle. As demonstrated by these results, proteasome inhibition by MG132 likely suppressed FTC236 growth by induction of cell cycle arrest.

DISCUSSION

The majority of patients with FTC have an excellent prognosis; however, a subset of these patients has aggressive, metastatic tumors. Currently, effective treatment options for these patients with metastatic FTC are needed. We sought to determine the effects of proteasome inhibition in human, metastatic FTC236 cells using the drug, MG132. FTC cell growth was profoundly suppressed by proteasome inhibition through the mechanism of cell cycle arrest (Figs. 2 and 3). Furthermore, inactive, phosphorylated GSK-3 β and active β -catenin expression were increased in FTC236 cells treated with MG132 (Fig. 1).

GSK-3 β is an important regulator of cell proliferation and apoptosis through its action as a serine/threonine protein kinase. GSK-3 β also plays a role in the phosphorylation of β -catenin

resulting in β -catenin degradation by the ubiquitin-proteasome system (8,9). An increase in inactive, phosphorylated GSK-3 β has been shown to suppress tumor growth in several cancers (7,15–17). In our experiments, phosphorylated GSK-3 β was not expressed in control cells suggesting that GSK-3 β is predominantly in its active form in FTC236 cells (Fig. 1). Moreover, with MG132 treatment, the amount of pGSK-3 β increased, while total GSK-3 β remained constant. Studies that have shown decreased tumor cell growth secondary to increased GSK-3 β phosphorylation also have seen no changes in the amount of total GSK-3 β by western blot analysis (15,17). These results suggest that the change in the amount of phosphorylated, inactive GSK-3 β underlies the mechanism of cell growth inhibition and not a change in total GSK-3 β , a ubiquitous cellular protein.

β -catenin and p β -catenin also were found to increase following treatment with MG132, representing accumulation of these proteins in the cells. Whether the large increase in β -catenin was due to proteasome inhibition or to changes in the levels of inactive, phosphorylated GSK3 β is unclear. Other tumor models have shown that phosphorylation of a single serine residue (Ser9) on GSK-3 β not only inhibits tumor growth, but also contributes to accumulation of active β -catenin in the cell (6,8,9). Alterations in the expression of p β -catenin may have occurred due to proteasome inhibition, since active GSK3 β leads to β -catenin phosphorylation. p β -catenin is then degraded by proteasomes in a ubiquitin-dependent manner. Therefore, the lack of proteosomal degradation caused by MG132 inhibition of the proteasome likely led to the minimal accumulation of p β -catenin. However, the results presented here do not provide enough evidence to draw a definite conclusion on the mechanisms of β -catenin and p β -catenin upregulation.

These data do indicate that MG132 is involved in the inactivation of the GSK-3 β pathway, which may be the mechanism of FTC236 growth inhibition. The cellular proliferation assay performed over 6 days showed a significant reduction in the growth of the human FTC236 cell line (Fig. 2). The effects of MG132 on GSK-3 β signaling could represent a direct and novel action of this drug or indirect effects of proteasome inhibition and build up of molecules upstream of the ubiquitin-proteasome system (UPS).

In this study, we also demonstrate that the mechanism of growth inhibition of MG132 appears to occur through cell cycle arrest. We observed an upregulation of p21^{Waf1/Cip1}, a member of the cyclin-dependent kinase inhibitor family, in response to MG132 treatment (Fig. 3). p21^{Waf1/Cip1} is known to be degraded through the UPS; therefore, increased levels of p21^{Waf1/Cip1} protein, following treatment with a proteasome inhibitor were expected (18). Additionally, β -catenin has been shown to act as a transcriptional regulator of p21^{Waf1/Cip1}, thus mediating G1 arrest and differentiation (19).

Levels of cyclin D1 also were reduced in the presence of MG132 proteasome inhibition. Cell cycle progression is facilitated by cyclin-dependent kinases, which are activated by cyclins, such as cyclin D1. In the cell cycle, cyclin D1 is specifically involved the transition from G1 to S phase (20,21). Furthermore, the changes in both cyclin D1 and p21^{Waf1/Cip1} protein levels suggest a deregulation of the cell cycle leading to its arrest. Previous reports indicate that GSK-3 β regulates expression of cyclin D1 through β -catenin suggesting that the growth inhibition of MG132 may result from its effects on GSK-3 β (21,22). These findings indicate that the mechanism responsible for inhibition of cellular proliferation of FTC cells treated with MG132 is cell cycle arrest.

The UPS plays a crucial role in the control of a number of cellular processes such as signal transduction, differentiation and development, cell cycle regulation, and apoptosis (12,23). The UPS is necessary for the highly selective turnover of intracellular proteins including tumor suppressors, transcription factors, and cell cycle proteins via their degradation (23,24).

Proteasome inhibitors have been shown to slow or even arrest cancer progression by interfering with the ordered, temporal degradation of regulatory molecules. Therefore, proteasome inhibitors are starting to be utilized in the treatment of various malignancies, including solid tumors (24,25). The conventional therapy used to treat FTC consists of thyroidectomy and radioiodine I^{131} treatment to ablate any remaining tissue following surgery (4). These approaches result in favorable long-term survival for the majority of patients; however, the prognosis is worse for patients with distant metastases. Aggressive, metastatic FTC cells often lose their capacity to take up iodine leading to resistance to therapeutic radioiodine, most likely due either to low expression of the hNIS gene or to hNIS dysfunction resulting from posttranscriptional events (4,5). Therefore, investigating alternative treatment options for these patients has become necessary (1).

This study demonstrated that proteasome inhibition by MG132 significantly suppresses the growth of a human, metastatic follicular thyroid cancer (FTC) cell line. MG132 treatment also altered expression of phosphorylated GSK-3 β and β -catenin in these cells suggesting that GSK-3 β signaling may be responsible for the growth reduction observed. In addition, we noted changes in the protein levels of the cell-cycle regulators, cyclin D1 and p21^{Waf1/Cip1}, which are known to be controlled by GSK-3 β through β -catenin. Taken together, these results support further investigation into MG132 as a possible alternative therapeutic strategy for patients with aggressive, metastatic FTC.

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References

1. Wang T, Roman S, Sosa J. Management of follicular tumors of the thyroid. *Minerva Chir* 2007;62:373–382. [PubMed: 17947948]
2. Carling T, Udelsman R. Follicular neoplasms of the thyroid: what to recommend. *Thyroid* 2005;15:583–587. [PubMed: 16029125]
3. Hundahl S, Cady B, Cunningham M, Mazzaferri E, McKee R, Rosai J, Shah J, Fremgen A, Stewart A, Hölzer S. Initial results from a prospective cohort study of 5583 cases of thyroid carcinoma treated in the united states during 1996. U.S. and German Thyroid Cancer Study Group. An American College of Surgeons Commission on Cancer Patient Care Evaluation study. *Cancer* 2000;89:202–217. [PubMed: 10897019]
4. Mazzaferri E, Kloos R. Clinical review 128: Current approaches to primary therapy for papillary and follicular thyroid cancer. *J Clin Endocrinol Metab* 2001;86:1447–1463. [PubMed: 11297567]
5. Venkataraman G, Yatin M, Marcinek R, Ain K. Restoration of iodide uptake in dedifferentiated thyroid carcinoma: relationship to human Na⁺/I⁻symporter gene methylation status. *J Clin Endocrinol Metab* 1999;84:2449–2457. [PubMed: 10404820]
6. Shakoori A, Ougolkov A, Yu Z, Zhang B, Modarressi M, Billadeau D, Mai M, Takahashi Y, Minamoto T. Deregulated GSK3 β activity in colorectal cancer: its association with tumor cell survival and proliferation. *Biochem Biophys Res Commun* 2005;334:1365–1373. [PubMed: 16043125]
7. Hardt S, Sadoshima J. Glycogen synthase kinase-3 β : a novel regulator of cardiac hypertrophy and development. *Circ Res* 2002;90:1055–1063. [PubMed: 12039794]
8. Liu C, Li Y, Semenov M, Han C, Baeg G, Tan Y, Zhang Z, Lin X, He X. Control of β -catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* 2002;108:837–847. [PubMed: 11955436]
9. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. β -catenin is a target for the ubiquitin-proteasome pathway. *EMBO J* 1997;16:3797–3804. [PubMed: 9233789]

10. Wu W, Sung J, Yu L, Cho C. Proteasome inhibitor MG-132 lowers gastric adenocarcinoma TMK1 cell proliferation via bone morphogenetic protein signaling. *Biochem Biophys Res Commun* 2008;371:209–214. [PubMed: 18435911]
11. Adams J. Proteasome inhibitors as new anticancer drugs. *Curr Opin Oncol* 2002;14:628–634. [PubMed: 12409653]
12. Adams J. The proteasome: a suitable antineoplastic target. *Nat Rev Cancer* 2004;4:349–360. [PubMed: 15122206]
13. Van Gompel J, Kunnimalaiyaan M, Holen K, Chen H. ZM336372, a Raf-1 activator, suppresses growth and neuroendocrine hormone levels in carcinoid tumor cells. *Mol Cancer Ther* 2005;4:910–917. [PubMed: 15956248]
14. Sippel R, Carpenter J, Kunnimalaiyaan M, Lagerholm S, Chen H. Raf-1 activation suppresses neuroendocrine marker and hormone levels in human gastrointestinal carcinoid cells. *Am J Physiol Gastrointest Liver Physiol* 2003;285:G245–254. [PubMed: 12851216]
15. Liao X, Thrasher J, Holzbeierlein J, Stanley S, Li B. Glycogen synthase kinase-3 β activity is required for androgen-stimulated gene expression in prostate cancer. *Endocrinology* 2004;145:2941–2949. [PubMed: 14988390]
16. Ougolkov A, Fernandez-Zapico M, Savoy D, Urrutia R, Billadeau D. Glycogen synthase kinase-3 β participates in nuclear factor kappaB-mediated gene transcription and cell survival in pancreatic cancer cells. *Cancer Res* 2005;65:2076–2081. [PubMed: 15781615]
17. Kunnimalaiyaan M, Vaccaro A, Ndiaye M, Chen H. Inactivation of glycogen synthase kinase-3 β , a downstream target of the raf-1 pathway, is associated with growth suppression in medullary thyroid cancer cells. *Mol Cancer Ther* 2007;6:1151–1158. [PubMed: 17363508]
18. Blagosklonny M, Wu G, Omura S, el-Deiry W. Proteasome-dependent regulation of p21WAF1/CIP1 expression. *Biochem Biophys Res Commun* 1996;227:564–569. [PubMed: 8878553]
19. van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der Horn K, Batlle E, Coudreuse D, Haramis A, Tjon-Pon-Fong M, Moerer P, van den Born M, Soete G, Pals S, Eilers M, Medema R, Clevers H. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* 2002;111:241–250. [PubMed: 12408868]
20. Walker J, Assoian R. Integrin-dependent signal transduction regulating cyclin D1 expression and G1 phase cell cycle progression. *Cancer Metastasis Rev* 2005;24:383–393. [PubMed: 16258726]
21. Hu D, Fang W, Han A, Gallagher L, Davis R, Xiong B, Yang W. c-Jun N-terminal kinase 1 interacts with and negatively regulates Wnt/beta-catenin signaling through GSK3 β pathway. *Carcinogenesis* 2008;29:2317–2324. [PubMed: 18952597]
22. Takahashi-Yanaga F, Sasaguri T. GSK-3 β regulates cyclin D1 expression: a new target for chemotherapy. *Cell Signal* 2008;20:581–589. [PubMed: 18023328]
23. Ciechanover A. The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J* 1998;17:7151–7160. [PubMed: 9857172]
24. Shah S, Potter M, Callery M. Ubiquitin proteasome pathway: implications and advances in cancer therapy. *Surg Oncol* 10:43–52. [PubMed: 11719028]
25. Wu W, Wu Y, Yu L, Li Z, Sung J, Cho C. Induction of autophagy by proteasome inhibitor is associated with proliferative arrest in colon cancer cells. *Biochem Biophys Res Commun* 2008;374:258–263. [PubMed: 18638451]

Figure 1.

Figure 2.

Figure 3.