UBS109, a novel curcumin analogue, promotes apoptosis in head and neck cancer cells through activation of the death receptor signaling pathway

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Abstract

Curcumin has gained increasing attention due to its potential therapeutic effects in a variety of diseases, such as cancer and inflammatory diseases. To improve its pharmacological properties, a derivative of curcumin, UBS109 (3,5-bis(pyridin-2-ylmethylene)piperidin-4-one), has been developed. Our study showed that UBS109 exhibited a potent anticancer activity, inhibiting colony formation and cancer cell growth *in vitro*, and tumor growth in a xenograft animal model *in vivo*. We also carried out mechanistic studies by profiling a panel of head and neck cancer cell lines. We found that UBS109 induced cell death in fourteen different head and neck squamous cell carcinoma cell lines with variable potency. In some cell lines, UBS109 was ten times more potent than curcumin. We demonstrated that UBS109 rapidly blocked the nuclear translocation of NF-κB, as expected with curcumin analogs. Further, we found that UBS109 potently suppressed the NF-κB signaling pathway through inhibition of AKT phosphorylation and then subsequent inhibition of IκB phosphorylation and degradation. Importantly, we discovered a novel mechanism by which UBS109 induces apoptotic death of TU212 cells through upregulation of the death-receptor signaling pathway. UBS109 effectively increased the level of FADD and accelerated the cleavage of caspase-8. These results, along with others, have led to a proposed study of UBS109 in a phase 0 clinical trial in head and neck cancer patients, which is expected to provide further insight into the development of the next generation of curcumin-based anticancer agents.

Introduction

Curcumin, a component of turmeric, has been shown to suppress tumor initiation, promotion, and metastasis by inhibiting nuclear factor kappaB (NF-κB), protein kinase C (PKC), mitogen-activated protein kinase (MAPK) and by activating protein-1 (AP-1) pathways. To improve the poor solubility of curcumin, we have synthesized a panel of monoketone curcumin analogues. One of them is UBS109. In order to identify candidate lead compounds for further clinical development, the cytotoxicity and mechanism of action of the curcumin analogue UBS109 were tested in head and neck cancer cells and a tumor xenograft mouse model.

Methods

Cell culture
All head and neck cancer cells were cultured in DMEM/F12 (1:1) supplemented with 10% FBS.
Cell viability assay
14 Head and neck cancer cells were plated in 1536-well plates and treated with curcumin or UBS109 at serially diluted concentrations for 72 hours. Next, an Alamar Blue assay was performed. The IC_{50} values were determined by using GraphPad Prism 5.0(San Diego, CA).
Colony formation assay
150 cells per well of TU212 cells were plated in 48-well plates and incubated overnight. Cells were treated with different concentrations of curcumin or UBS109 for 10 days. Formed colonies were stained with SRB 10 days after treatment.
NF-κB translocation assay
2000 TU212 cells were seeded in each well of 1536-well plate and incubated overnight at 37 ℃. Cells were exposed to TNFα (60ng/ml) for 20min after treatment with compounds for 30 minutes. Cells were fixed with 2% paraformaldehyde and permeated with 0.1% TritonX-100. NF-κB was detected with antibodies against p65 and Alexia fluor 488 conjugated anti-rabbit IgG. The plates were scanned and the images were quantified by using ImageXpress 5000 A (Molecular Devices).
Flow cytometry analysis
Apoptosis was examined by measuring percentage of annexin V-PE -stained cells. TU212 cells were treated with curcumin or UBS109 for 24 hrs. Attached and unattached cells were washed with 1xPBS and then digested into single cell. AnnexinV-PE was used to stain live cells for 15min, and the percentage of positive stained cells were measured by a FACScan cytometer.
Western blotting
Cells were lysed in 1.0% NP-40 buffer. Equal volumes of cell lysate were subject to electrophoresis on 12.5% SDS-PAGE. Proteins were then electrotransferred to a nitrocellulose membrane. The membranes were incubated with primary antibody and then the corresponding horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG as indicated.
In vivo xenograft model
Athymic nude mice (Harlan, 5 weeks old, female) were housed in a pathogen-free facility, and all procedures and protocols were approved by the Institutional Animal Care and Use Committee at Emory University. TU212 cells (2 × 10^6 cells/0.1ml HBSS and 0.015ml matrigel matrix/ mouse) were inoculated subcutaneously into the left axillary mammary fat pad of mice and allowed to form a xenograft. When tumors reached a volume of approximately 250 mm^3, mice were randomized to receive either vehicle [0.5% Carboxymethyl Cellulose Sodium (CMC) with 10% DMSO in sterile water] or UBS109 with different dosages. UBS109 was administered by oral gavage 5 days per week at a dosage of 25, 50, 100, or 150 mg/kg/treatment. Body weights were checked everyday and tumor size measured two or three times a week. Tumor volume was calculated using the formula (width² × length)/2.

Results

Curcumin analogue UBS109 exhibits more potent cellular toxicity in head and neck cancer cell lines than curcumin

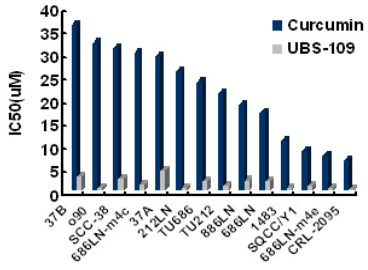

Figure 1. The effect of curcumin and UBS109 on the viability of 14 head and neck cancer cell lines. Head and neck cancer cells were grown in 1536-well plates and were treated with serial dilutions of curcumin or UBS109 for 72hrs. Cell viability was assessed by the Alamar Blue method. The IC_{50} value was obtained from a dose-response curve and plotted by using GraphPad Prism5 software.

UBS109 induces apoptosis and inhibits colony formation of TU212 cells in a dose-dependent manner

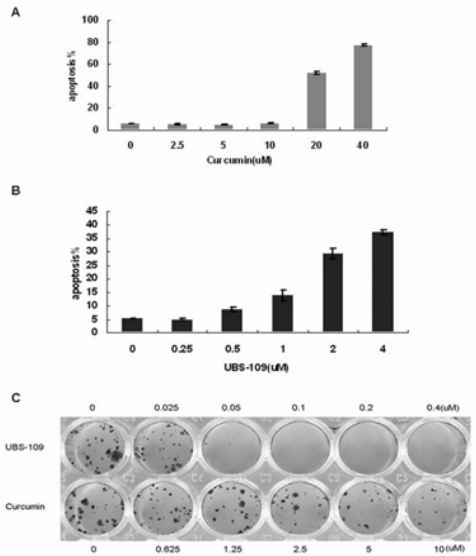

Figure2. The effect of treatment with curcumin or UBS109 on apoptosis and colony formation in TU212 cells. Cells were exposed to curcumin (A) or UBS109 (B) for 24 hrs. Flow cytometry was performed to define the percent of cells in apoptosis based on annexinV-staining as described in Materials and Methods. The error bars indicate the standard deviation of the mean of 3 independent samples. (C) A colony formation assay was performed with TU212 cells in the presence of curcumin or UBS109 at the indicated concentrations.

UBS109 inhibits tumor growth in a TU212 xenograft model

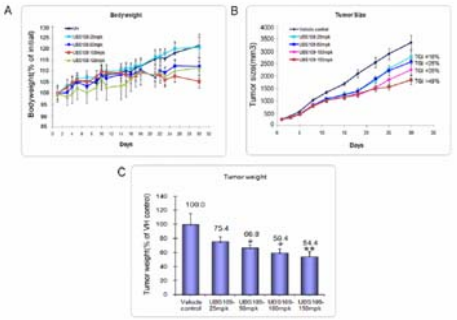

Figure 3. UBS109 inhibits tumor growth in the TU212 xenograft model. A) A graph representing the body weight of mice with TU212 xenografts during the USB109 treatment period. The body weight of mice with TU212 xenografts did not change significantly with dose of UBS109. B) A graph representing tumor size during USB109 treatment. Tumor size was measured two or three times a week using a digital caliper (Fisher Scientific). The treatment groups show tumor growth inhibition (TGI) in a dose-dependent manner. C) Tumor weight for different doses of USB109 at autopsy. The decrease in tumor weight is statistically significant at a dose of 50 mg/kg of UBS109 and above. Additionally, the inhibition of tumor weight correlated with the observed decrease in tumor size. The number of mice in each group was 8. A student t-test was used for the statistical analysis. *: p<0.05, **:p<0.01.

UBS109 blocks TNF-α induced NF-κB nuclear translocation by inhibiting AKT phosphorylation

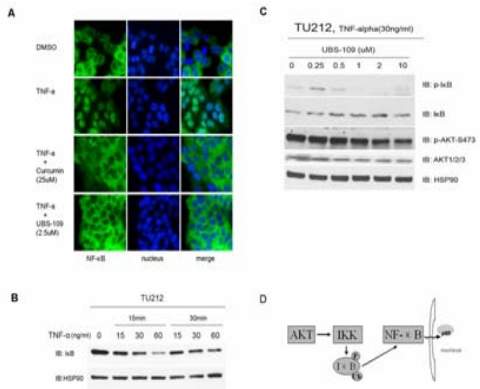

Figure 4. Effect of UBS109 on TNF-α induced NF-κB nuclear translocation. (A) TU212 cells were grown in 1536-well plates and treated with TNF-α or control (DMSO) for 30 min prior to NF-κB detection, as described in the methods. (top two rows of A). The effect of UBS109 or curcumin treatment (30 min) on TNF-α -induced nuclear translocation of NF-κB (lower two rows in A). (B) Multiple doses and times of TNF-α treatment in TU212 cells were examined for NF-κB translocation. An antibody against the IκBα protein was used to detect NF-κB translocation. (C) TU212 cells were treated with UBS109 for 30 min prior to TNF-α stimulation. Cell lysates were collected and the status of AKT phosphorylation, IκBα phosphorylation and degradation were determined by using phospho-specific antibodies. (D) Illustration of a proposed mechanism for UBS109 inhibition of NF-κB nuclear translocation.

UBS109 induces apoptosis in TU212 cells by up-regulating the death-receptor signaling pathway

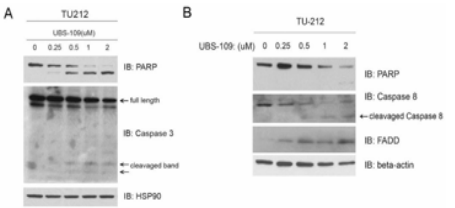

Figure 5. Mechanism of UBS-109 induction of TU212 apoptosis. (A) TU212 cells were treated with UBS109 at the indicated concentrations. Cell lysates were collected to detect apoptosis, as indicated by marker proteins PARP and Caspase 3. HSP90 was included as a protein loading control. (B) Additional apoptotic proteins, cleaved caspase 8 and FADD, were detected under the same treatment conditions. Beta-actin was included as a protein loading control.

Figure 6. Proposed mechanism for UBS109 induction of apoptosis in TU212 cells. Our results support a model in which UBS109 upregulates the death-receptor signaling pathway.

Conclusion

1. UBS109, a new curcumin analogue, has cellular effects similar to curcumin. UBS109 more potently promotes head and neck cancer cells to undergo apoptosis than curcumin. UBS109 was slightly more potent with metastatic head and neck cancer cell lines, based on UBS109 IC_{50} values among several pairs of low-high metastatic content cell lines: 37A VS 37B; TU212 VS 212LN; FaDu VS CRL-2095 (data not shown).
2. Inhibition of tumor growth in an *in vivo* xenograft animal model strongly supports UBS109 as a potential new therapy for head and neck cancer.
3. UBS109 rapidly blocks the NF-κB signaling pathway through the inhibition AKT phosphorylation and the subsequent inhibition of IκB phosphorylation and degradation.
4. UBS109 promotes apoptosis in TU212 cells by up-regulating the death-receptor signaling pathway.

Reference

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