Specifically Binding of D-Amino Neuraminic Acid to Ganglioside Studied in Prostate Cancer Cells

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Abstract

Aims: The aim of this study was to investigate the cellular binding site of human KDN (2-keto-3-deoxy-D-glycero-D-galacto-nononic acid). The KDN molecule is a member of the sialic acid family, and its expression increases in cancer cells. Although KDN has been shown to bind to GM3 (Monosialodihexosyl Ganglioside) in trout sperm.

Methods and Results: Prostate cancer cell line (DU145) was used in this study. Each experimental group was divided into 3 groups: control, GCS (Glucosylceramide synthase) enzyme inhibitor Genz-123346 treated, and GM3 synthesis inhibitor Triptolide treated. Each group was stained using the immunocytochemical method for GM3, GD3 (Disialosyllactosylceramide) and KDN. The FTIR (Fourier Transform Infrared Spectroscopy) analysis was performed to elucidate the cellular changes upon treatment. The non-treated number 1 cell group stained positive with all of GM3, GD3 and KDN, the GCS enzyme blocked with Genz-123346 number 2 cell groups stained positive with only KDN. Furthermore, GD3 Synthase inhibitor Triptolide treated number 3 cell group stained positive with GM3 and KDN. Measurements with FTIR showed apoptotic features with Triptolide while Genz-123346 had no negative effect on the cell viability. The decrease in sugar constructions was revealed and the results that we obtained with staining were reinforced.

Conclusions: Determining the location of bounded KDN is important in selecting new targets for cancer treatment researches. It has been shown that KDN is not inhibited by both GM3 inhibition and GD3 inhibition, and thus, KDN might also bind to different places, be specific, and not only attached to any of gangliosides of the GM or GD series.

Introduction

Malignant tumors consist of cancer cells with different morphological characteristics. Cancer cells express varying cell surface markers for each type of tumor and display intratumoral heterogeneous populations with different proliferation capacities. This heterogeneity is due to genetic/epigenetic and micro-environmental differences as well as due to different levels of differentiation. In targeted cancer therapy, identification of surface antigens, signalling pathways, genetic landscape and intercellular connection complexes are very important. Differentially expressed, novel proteins, carbohydrates and lipid structures in cancer cells, are promising targets for the development of new therapeutical strategies.

Recent studies have shown that various glycolipids play a role in cancer cell invasion, neovascularization, and immune escape during metastasis [1, 2]. Blockage of glycolipids such as GM3 and GM2 has been shown to reduce tumor cell metastasis and to induce apoptosis in cancer cells [3]. Gangliosides are sialic acid-containing glycosphingolipids, and recently, some cancers have been shown anti-cancer effects by blocking ganglioside molecules [4].

Gangliosides are abundant in the brain tissue, where they occur 5-fold higher in the grey matter when compared to the white matter. In extraneural tissues, the ganglioside content is lower than in the brain;

and can be in relatively higher concentrations in bone marrow, erythrocytes, intestine, liver, spleen, and testis, kidney, and embryonic stem cells. Gangliosides may act as receptors for certain hormones and bacterial toxins [5]. They are involved in cell adhesion/recognition processes, signal transduction, tissue growth and differentiation. Their composition is known to change in different pathologies including carcinogenesis [5]. Gangliosides are involved in intracellular membrane structures such as the nuclear envelope [6]. Defects in ganglioside metabolism due to genetic mutations in lysosomal enzymes responsible for their degradation result in accumulation in the musculoskeletal system, liver, spleen and central nervous system, giving rise to a group of diseases called sphingolipidosis [7].

Gangliosides are synthesized from ceramide and contain one or more sialic acid residues. The structure consists of a glycosphingolipid (ceramide and oligosaccharide) and one or more N-acetyl neuraminic acid residue. The synthesis of ganglioside starts with the conversion of ceramide by GCS (Glucosylceramide Synthase) to glucosylceramide (Glc-Cer). Glucosylceramide is converted to lactosylceramide (Lac-Cer, Gal-Glc-Cer) by galactosyltransferase I. Monosialodihexosyl ganglioside (GM3) is synthesized from lactosylceramide by GM3 synthase enzyme. Other gangliosides are synthesized via GM3. The GM2 is synthesized from GM3 with the GalNAc-T (B4GALNT1, Beta-1,4 N-acetyl galactosaminyl transferase 1) enzyme while disialosyllactosylceramide (GD3) is synthesized from GM3 by GD3S (GD3 Synthase, Alpha-N-acetyl neuraminide alpha-2,8-sialyl transferase). The GM2 is synthesized from GM3 and GM1 from GM2. GM3 is the key molecule for the synthesis of other gangliosides [8]. It has been shown in several studies that the synthesis of GM3 -namely all gangliosides- can be successfully inhibited by various molecules during the conversion from ceramid in the GCS step [9]. The main molecules used in enzyme inhibition are Genz-123346, PPPP, PMPP, PDMP, NB-DNJ (N-butyl-deoxynojirimycin) and Genz-529468 [9]. The GD3 synthesis by GD3 synthase can be blocked by Triptolide [1].

2-Keto-3-deoxy-D-glycero-D-galacto-nonanic acid (KDN) is considered a member of the sialic acid family with unique features. KDN is formed by the addition of a hydroxyl group to the C5 position in the common Sia, instead of the aceto-amide group. KDN has different properties, including insensitivity to various sialidases and a "stop signal" for the prolongation of polysialic acid. In mammalian cells, KDN is often expressed at very low levels. It was first isolated by Inoue et al. as a polysialo-glycoprotein [10]. The KDN molecule is synthesized by two separate pathways: 1- synthesis by de-acetylation from Neu5Ac; 2- de novo synthesis by different enzymes. KDN is found in many animals, from bacteria to high vertebrates including humans. KDN has been shown to date in the trout spore based on GM3 [11]. No further studies have been conducted on KDN. The putative role of KDN in cancer can be demonstrated by the elucidation of its chemical structure. The aim of this study was to demonstrate whether the KDN is only associated with GM series gangliosides.

Materials And Methods

Cell culture

Prostate cancer cell line (DU145) was obtained from American Type Culture Collection (ATCC). RPMI 1640 medium was used for the growth and maintenance of DU145 cell line. 1% Penicillin/streptomycin, 10% heat inactivated Fetal bovine serum, 1% amphotericin B and 1% L-glutamine were added in RPMI 1640 medium. All cells were grown at 37°C, 5% CO₂. Cell line was monitored daily in terms of viability, proliferation and infection by inverted microscope. When more than 80% cell density was observed, the cells were propagated by passaging.

Cell Count and Viability Assay

Viability of the cells following treatment was determined using the Muse™ Count and Viability kit (Muse™ Cell Analyzer; Millipore, Billerica, MA, USA) according to the manufacturer's instructions. Briefly, cells were seeded in triplicate in 6-well plates at a density of 1×10⁴ cells/well. After 24 hours incubation, cells were exposed to increasing concentrations of Genz-123346 (10, 100, 1000 nM) and Triptolide (20, 40, 60 nM). Then, the plates were incubated at 37°C in a 5% CO₂ incubator for 24, 48, and 72 h. After incubation, all cells were collected and diluted with phosphate buffered saline (PBS). 50 µl of cell suspension was then added into 450 µl Muse Count and Viability reagent (10x dilution), incubated for 5 min at room temperature, and analyzed using the Muse Cell Analyzer. Data were presented as proportional viability (%) by comparing the treated group with the untreated cells, the viability of which is assumed to be 100%.

Immunocytochemical Staining

Cells were placed on coverslips and fixed with 4% paraformaldehyde for 30 minutes at 4°C and permeabilized with 0.01% Triton X-100 for 15 minutes at room temperature. Cells were blocked serum blocking solution (Invitrogen, USA) for 30 min and incubated with primary antibodies (dilution of 1:100, at 4°C overnight) Anti-KDN antibody, Anti-Ganglioside GD3 antibody (ab11779) and Anti-GM3 Monoclonal antibody (AMS.A2582) in a humidity chamber. After washing with PBS, cells were incubated with secondary antibody and streptoavidin complex for 30 min. Substrate solution (DAB) (Invitrogen, USA) was applied, and then, Mayer's hematoxylin was used for counter staining. Immunoreactivity of molecules was assessed by light microscopy using Olympus BX-51 and C-5050 digital cameras.

Fourier Transform Infrared (FTIR) Spectroscopy

i. Sample Preparation

The cells were grown in T25 flasks. The cells were separately treated with GCS inhibitor Genz-123346 at 1000nM for 24 hours and GD3s inhibitor Triptolide at a 60nM dose for 72 hours. Before harvesting the cells, the medium was discarded and the cells were rapidly washed with 1ml 0.05% trypsin (Sigma Aldrich) to remove the dead cells debris. Then, the cells were detached from their culture support applying a 5 min-treatment with 1ml of trypsin buffer at 37°C in a 5% CO₂ environment. The reaction was stopped by rinsing the cells with 5ml of culture medium with 10% FBS (Fetal bovine serum). The cells were pelleted by a 2 minute centrifugation (300 g), and washed three times with sterile isotonic solution (NaCl,

0.9%) to ensure the removal of trypsin and the culture medium completely. Finally, the cells were then resuspended in the NaCl solution for the ATR-FTIR analysis. Three independent cultures of four different conditions were grown for each condition, as given below:

- DU145 (untreated prostate cancer cell lines, called as control group)
- DU145 + Genz (Genz treated prostate cancer cells)
- DU145 + Triptolide (Triptolide treated prostate cancer cells)

ii. ATR-FTIR Spectroscopy Measurements

The measurements were performed with an IRTracer-100 FTIR spectrometer (Shimadzu, Japan) combined with an attenuated total reflection (ATR) unit and equipped with a DLATGS detector, as described in our previous studies [12–14]. After a 2 μ l amount of cell suspension ($\sim 1 \times 10^6$ cell/ml) was placed on ATR, it was evaporated for ~ 12 min to get rid of the excess water in the cell suspensions. A total of 128 scans were averaged for each interferogram at 4 cm^{-1} spectral resolution. The spectrum of air was recorded as background spectrum in the absence of cell sample when the ATR plate was empty. Three samples taken from each condition were measured so that the measurements were triplicated. A minimum of four spectra per each measurement were recorded in the range of $4000\text{--}800 \text{ cm}^{-1}$.

iii. FTIR Data Processing

Spectral preprocessing, FTIR-difference spectra and Student's t-tests were performed by using the software 'Kinetics' running under MATLAB, as described in detail in our previous studies [12–14].

Spectral Preprocessing – After the IR spectra were recorded with the software *LabSolutions* (Shimadzu, Japan), the spectral preprocessing and visualization of the data were performed with the *Kinetics* program. The all recorded spectra for each cell condition at different times should be comparable, thus these data require preprocessing prior to further data analysis (employing of difference spectrum, mean spectrum etc). Accordingly, first, the contribution the atmospheric water vapor in the IR spectra was subtracted by taking the $1562\text{--}1555 \text{ cm}^{-1}$ as reference peak. Afterwards, the straight lines were interpolated between the points of the spectrum at the multiple wavenumbers. Then, they were subtracted from the spectrum for baseline-correction. After baseline correction, the spectra were normalized for the equal area between 1582 and 1482 cm^{-1} . Thus, the absorbance scale displays the re-scaled absorbance values in figures. The 'mean' absorbance spectrum was also calculated for each cell condition by averaging the all (baseline corrected and normalized) spectra recorded for triplicate experiments for each cell condition (grown independently). This was called as 'mean' in the present work.

Infrared Difference Spectra and Student's t-Test- The difference spectra were calculated using the mean absorbance spectra. For this purpose, the mean absorbance spectrum of 'untreated control DU145 cells' was subtracted from the mean absorbance spectrum of 'treated cells with Genz (or with Triptolide)', represented as follows: $\Delta A = (\text{treated}) - (\text{untreated})$. Student's t-test was also employed at every wavenumber, allowing a statistical comparison between the spectra of each cell conditions. Herein, the

red points demonstrate the wavenumbers where a significant difference exists with a significance $\alpha = 0.1\%$.

Results

Cytotoxic Effects of Drugs

Our purpose in the Muse analysis is to find out the IC-50 value of drugs (GCS Inhibitor Genz-123346 and GD3S Inhibitor Triptolide) used in the present study.

The effect of GCS inhibitor on the cell viability in DU145 cells was studied by applying the increased dose (at 10-1000 nM) and the time (at the 24, 48 and 72 hours).

At the end of 24, 48 and 72 hours treatment with GCS inhibitor, no significant cytotoxic effects were determined in the dose range of 10-1000 nM in DU145 cell line compared to the control. Genz-123346 was not cytotoxic on the cells. At the end of 72 hours, 95.9% of DU145 cell line was found in the dose of 1000nM Genz. As a result, the IC-50 value of Genz-123346 was not found in DU145 cell line. The cytotoxic effect of the Genz-123346 concentrations of 10, 100, 1000nM on the DU145 cell line (at 24, 48 and 72 hours) at various times (24, 48 and 72 hours) was not statistically significant (Figure 1a).

The effect of GM3 inhibitor on the cell viability of DU145 in regard to both increased dose (20-60 nM) and the time (24, 48 and 72 hours) was also investigated.

After 24, 48 and 72 hours of treatment with the GM3 inhibitor, a significant cytotoxic effect was determined in the DU145 cell line when the Triptolide concentrations of 20-60 nM were compared with the control group. At the end of 72 hours, IC-50 values were determined as 60nM dose of Triptolide. The cytotoxic effect of the Triptolide concentrations of 20, 40 and 60 nM on the DU145 cell line of GD3S inhibitor (24, 48 and 72 hours) was found statistically significant (Figure 1b).

Immunocytochemical Staining

GCS Inhibitor Genz-123346 Treated Cell Lines

Changes of GM3, GD3 and KDN in the cell lines treated with GCS inhibitor were determined by immunocytochemistry staining method. Cells were treated with GCS inhibitor at 1000nM for 24 hours. The GCS inhibitor was not added to the control group. When compared with the control group without GCS inhibitor, a significant decrease in immunocytochemistry staining with GM3 and GD3 antibodies was observed in the DU145 cell line after the Genz-123346 staining.

Although Genz-123346 inhibited the GM3 and GD3 gangliosides, the KDN was not inhibited (Figure 2).

GM3S Inhibitor Triptolide Treated Cell Lines

Changes in GM3, GD3 and KDN in DU145 cell lines treated with GD3S inhibitor were determined by immunocytochemistry staining method. Cells were treated with the GD3S inhibitor at a 60nM dose for 72 hours. No GM3 inhibitor was added to the control group. There was a significant decrease in GD3 in immunocytochemical staining after Triptolide in DU145 cell line compared to the control group without GM3 inhibitor.

The GM3 ganglioside and the KDN were not inhibited despite the inhibition of GD3 ganglioside in ICC staining with GD3S inhibitor Triptolide (Figure 3).

FTIR spectroscopy results

Our main purpose in the FTIR analysis is to examine the effects of the drug on cellular molecules (protein, lipid, carbohydrate, nucleic acid) in prostate cancer cells. Apoptotic spectral features were not observed by application of Genz-123346 in the DU145 prostate cancer cell line. Therefore, only the changes due to GM3 blockade were observed. An increase in the

α -helical proteins (1656 and 1549 cm^{-1}) was detected in the prostate cancer cell line after Genz-123346 treatment (the Amide I and Amide II bands have positive absorbance peaks). A more increase in the lipid signals was observed in the case of Genz administration when compared to the tryptolide administration (unsaturated $=\text{CH}$ at 3011 cm^{-1} , saturated CH_2 at 2922 and 2853 cm^{-1} , CH_2 bending at 1460 cm^{-1} and $\text{C}=\text{O}$ stretching at 1745 cm^{-1}) (for band assignments see ref [15, 16]). In our study, both sialic acid signals (1118 , 1044 and 1011 cm^{-1}) and the carbohydrate (sugar derivatives) signals (the $\text{C}-\text{O}$ stretching and $\text{C}-\text{O}-\text{H}$ bending modes of glycosidic bonds at $1150\text{-}950\text{ cm}^{-1}$) were also decreased (Figure 4) (for band assignments see ref [21, 22]).

Regular secondary structures of prostate cancer cell line proteins was reduced upon application of Triptolide in the DU145 cell line (the Amide I and Amide II bands have negative absorbances): protein α -helix structures (1659 cm^{-1}) decreased while β -sheets (1629 cm^{-1}) increased. This strongly suggests that apoptosis occurs in the cell line after treatment.

After Triptolide treatment, the increase in the lipid signals was also observed (unsaturated $=\text{CH}$ at 3013 cm^{-1} , saturated CH_2 at 2922 and 2853 cm^{-1} , CH_2 bending at 1464 cm^{-1} and $\text{C}=\text{O}$ stretching at 1741 cm^{-1}). This spectrum is most likely due to apoptosis-induced plasma membrane changes, which also results in the reduction of gangliosides, sugar derivatives (oligosaccharides, monosaccharides) and sialic acids. The carbohydrate (sugar derivatives) signals ($1180\text{-}900\text{ cm}^{-1}$) and sialic acid signals (1118 , 1044 and 1011 cm^{-1}) decreased significantly as well (Figure 4).

The FTIR results support the immunocytochemical staining results obtained herein.

Discussion

Cancer cells use gangliosides and some other glycolipids for making angiogenesis, invasion, metastasis and escaping the immune system [1, 2].

Blockage of glycolipids has been tried as a treatment and the blocking of some glycolipids has been shown to reduce tumor cell metastasis and it has even been found to lead the cancer cell to apoptosis [3] Genz-123346 has been used in a few studies for inhibition of GCS [17, 18].

In 2010, in Natoli and Karman's study, Mice were treated with Genz-123346. It showed the effects of treated mice's kidneys. This *in vivo* study showed that both GSC and GM3 were reduced by immunohistochemical and Western blot methods with Genz-123346 [17]. Karman has found the same result by using Western Blot Method in mouse lung [18]. Similar results were obtained in DU145 human prostate cancer cell line culture in our study. In Haw-Young Kwon's study, inhibition of GD3 by Triptolide was demonstrated by a decrease in hST8Sia I gene expression in SK-MEL-2 human melanoma cells [19]. In our study, inhibition of GD3S in the DU145 cell line by Triptolide was demonstrated by the immunocytochemical staining and FTIR spectroscopy. The relationship between KDN and GM3 was shown by Song Yu by Thin Layer Chromatography (TLC), Western Blot and immunoelectron microscopy [11].

In our study, we wanted to determine whether KDN is specifically associated or not to GM3. However, according to the immunocytochemical staining and the FTIR results we did not reject GM3 relationship, but it was revealed that it would not be possible to talk about GM3 specifically. Increased ganglioside levels can be used to evaluate tumor prognosis in patients with bladder cancer [20]. In a study, the association of GD3 ganglioside with Snail, Twist, and TGF- β 1 in epithelio-mesenchymal transition and metastasis in breast cancer was demonstrated. *In vitro* SUM159 and MDA-MB-231 in the study of cell lines, GD3 is required for wound healing, invasion and stem cell function. In the *In vivo* mouse studies, the inhibition of GD3S inhibits the metastasis [12]. It has been shown that gangliosid GM3 is related to Cisplatin-induced apoptosis in human colon cancer. It has been found that the reduction of GM3 synthase with siRNA and hence all other gangliosides, especially GM3, precursors protect HCT116 human colon carcinoma cells from Cisplatin-mediated apoptosis and apoptotic signal pathways due to free oxygen radical damage such as Bax, Bcl-2 and p53 have been shown to be inhibited [4].

In the FTIR analysis, apoptosis was not observed upon Genz-123346 administration. Therefore, only the changes due to GM3 blockade were observed. In the prostate cancer cell line, α -helix structural proteins were observed to increase at 1656 and 1549 cm^{-1} (the Amide I and Amide II bands have positive absorbances). The lipid signals show a greater increase when compared to the application of Triptolide (unsaturated =CH at 3011 cm^{-1} , saturated CH_2 at 2922 and 2853 cm^{-1} , CH_2 bending 1460 cm^{-1} and C=O at 1745 cm^{-1}). This sequence of spectra reflects the exposure of lipid fatty acids to the cell surface as a consequence of the reduction in the gangliosides and sugar derivatives (oligosaccharides, monosaccharides) and sialic acids on the extracellular surface after the treatment of Genz-123346, which blocks the synthesis of GM3 synthesis. This shows that the plasma membrane fatty acids are significantly uncovered. The reduction in the 1616 and 1514 cm^{-1} IR signal (arising from C-C bond

vibrations of carbohydrates) is most likely due to the low amount of carbohydrates, sugar derivatives and sialic acids. Due to a significant reduction in the amount of negatively charged sialic acids after treatment with genz, negatively charged COO^- molecular group signals ($1616\text{-}1576$ and 1393 cm^{-1}) were also reduced.

In our study, it was shown that carbohydrate (sugar derivatives) signals arising from the C-O stretching and C-O-H bending vibrations of glucosidic bonds absorbing at $1150\text{-}950\text{ cm}^{-1}$ [21] as well as the sialic acid signals (1118 , 1044 and 1011 cm^{-1}) [22] were also decreased.

When Triptolide was applied, the regular structures of prostate cancer cell line proteins are reduced. Protein α -helix structures decreased at 1659 cm^{-1} while β -sheet structures increased at 1629 cm^{-1} (negative absorbance at the Amide I and Amide II bands). This strongly suggests that apoptosis occurs after treatment [23].

Due to the application of Triptolide, an increase in lipid signals is observed. The lipid signals are increased at 3013 cm^{-1} (unsaturated $=\text{CH}$), 2922 and 2853 cm^{-1} (saturated CH_2), 1464 cm^{-1} (CH_2 bending mode) and at 1741 cm^{-1} (ester $\text{C}=\text{O}$), respectively. This pattern of spectrum is most likely due to the apoptosis-induced plasma membrane blebbing, which also results from the reduction of gangliosides, sugar derivatives (oligosaccharides, monosaccharides) and sialic acids. The negatively charged COO^- molecular group signals are also decreased ($1595\text{-}1576$ and $1415\text{-}1397\text{ cm}^{-1}$), probably due to a significant reduction in the amount of negatively charged sialic acids and amino acids. In the spectral region between 1250 and 1200 cm^{-1} , the phosphate groups of the DNA (phosphodiester vibrations) give rise to peaks, and the decrease in this signal indicates that the IR signals from DNA are also reduced. This indicates the DNA fragmentation due to apoptosis [24, 25].

In our study, the results of FTIR analysis, ICC staining results and Muse IC-50 results support each other. Genz-123346 did not show any cytotoxic effect on cells and no IC-50 value was found in Muse assay. The same result was demonstrated by showing Genz-123346 that did not induce apoptosis in the DU145 cell line based on the FTIR analysis. In the FTIR analysis of Triptolide, it was shown that the DU145 cell line underwent apoptosis, with the result of the IC-50 study in the Muse assay, the compatible result has arisen. The reduction in the amount of sialic acid in the DU145 cell line by the application of Genz and Triptolide in the FTIR analysis coincides with ICC staining. In this study, it was determined whether GDN is specifically dependent on or not the GM3. However, according to the results of immunocytochemical staining we did and the FTIR analysis, GM3 does not reject the relationship but it is not possible to mention any specific attachment to GM3. With the results of the study, the KDN has not been shown on GM3 but has led to another discussion. KDN is not only present on GM3 but may also be present on free or bound to other structures on cellular membranes. It can not be concluded from this study that GDN is not present on GM3, but it can be concluded that the only location is not only GM3.

Declarations

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Compliance with Ethical Standards

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Research involving human participants and/or animals

Not available, no human or animal study has done.

Informed consent

Not available, no human study has done.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Data Availability Statement

Authors can confirm that all relevant data are included in the article and/or its supplementary information files.

Authors' Contribution

The authors confirm contribution to the paper as follows: Study conception and design: Demir K; data collection: Demir K, Yigitturk G, Acikgoz E, Guler G, Rouhrazi H; analysis and interpretation of results: Demir K, Yigitturk G, Acikgoz E, Guler G, Aktug H, Oktem G; draft manuscript preparation: Demir K; All authors reviewed the results and approved the final version of the manuscript.

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Figures

Figure 1

a. The effects of GCS inhibitor Genz-123346 on cell viability, DU145 cell line on cell viability at 24, 48 and 72 hours. b. The effects of the GD3S inhibitor Triptolide on cell viability at 24, 48 and 72 hours in the DU145 prostate cancer cell line.

Figure 2

GCS inhibitor Genz-123346 treated with 1000nM dose for 24 hours. In the DU145 cell line a. GM3 ganglioside, b. GD3 ganglioside and c. Light microscopic image of the change in KDN (40X).

Figure 3

Treatment of GD3S inhibitor Triptolide by 72nM for 72 hours. DU145 cell line a. GM3 ganglioside, b. GD3 ganglioside and c. Light microscopic image of the change in KDN (40X).

Figure 4

a. A series of the FTIR spectra for DU145 human prostate cancer cells treated with control, Genz-123346 and Triptolide in the spectral region of 3100-2800 and 1800-800 cm^{-1} . b. The FTIR-difference spectra (obtained from Figure 4a). Each Triptolide and Genz-123346 administered FTIR data were shown in the 3100-2800 cm^{-1} and 1800-800 cm^{-1} ranges. The spectra were baseline corrected and normalized to the amide II band.