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ARTICLE

1-Deoxynojirimycin Inhibits Metastasis of B16F10 Melanoma Cells by Attenuating the Activity and Expression of Matrix Metalloproteinases-2 and -9 and Altering Cell Surface Glycosylation

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1-Deoxynojirimycin (1-DNJ), an iminosugar rich in mulberry, has been shown to possess antimetastatic potential. The antimetastatic mechanisms of 1-DNJ in melanoma B16F10 cells were studied, as were the antimetastatic activity (cell adhesion, migration, and invasion) of 1-DNJ, matrix metalloproteinases (MMP-2 and MMP-9), tissue inhibitors of metalloproteinase (TIMP-1 and TIMP-2) mRNA, and flow cytometric analysis of cell surface in melanoma B16F10 cells. 1-DNJ significantly inhibited invasion, migration, and cell-matrix adhesion and markedly decreased MMP-2 and MMP-9 activity and mRNA expression. In contrast, 1-DNJ effectively enhanced the expression of TIMP-2 mRNA. In addition, 1-DNJ significantly decreased abnormal glycosylation and/or sialylation on B16F10 melanoma cell surface but increased the levels of α-mannose. Thus, the antimetastatic effects of 1-DNJ against B16F10 melanoma cells are likely associated with its attenuated activities and expression of MMP-2/9, enhancement of the TIMP-2 mRNA expression, and alterations of the cell surface-binding motif. These results suggest that 1-DNJ may be useful as an adjuvant of antimetastatic agents such as cisplatin.

KEYWORDS: 1-Deoxynojirimycin (1-DNJ); antimetastatic potential; matrix metalloproteinases-2 and -9; cell surface glycosylation

INTRODUCTION

Cancer metastasis is one of the major challenges in cancer research. Cancer metastasis occurs via a complex biochemical multistage that enables carcinoma in situ to spread and colonize secondary sites. At the cellular level, cancer cells move by their own motility, and control of the migration or invasion of cancer cells is an important problem in tumor treatment (*1*). Recently, much attention has been drawn to the physiological relevance of matrix metalloproteinases (MMPs), which play important roles in the invasive ability and malignancy of tumor cells (*2*). In addition, the balance between MMPs and tissue inhibitors of metalloproteinases (TIMPs) seems to determine the metastatic capacity of melanoma cells both in vitro and in vivo (reviewed in ref *3*). MMPs play an important role in extracellular matrix (ECM) degradation, whereas TIMPs are the specific regulators of MMPs. MMP-2 and MMP-9 of MMPs are key enzymes in cancer cell metastasis, resulting from collagen degradation, and expression of MMP-2 and MMP-9 mRNA has been associated with a higher incidence of cancer metastasis (*4*). It has been shown that expression of MMP-2 is increased in advanced phases and that expression of MMP-9 is limited to early phases of melanoma, indicating that MMP-2 and MMP-9 are involved in degradation of the ECM during different stages of cancer progression (*3*). TIMPs, such as TIMP-1, -2, -3, and -4, can regulate or inhibit MMPs activity and are thus involved in cell invasion and metastasis (*5*). During tumor metastasis the balance of MMPs/TIMPs is broken, favoring ECM degradation without regulation. Indeed, it has been shown in melanoma metastasis that patients with a form of cancer that expresses TIMPs have a better survival rate (*6*).

Tumor cell-surface oligosaccharides have been shown to be remarkably distinct from those of normal cells (*7*). In cancer biology, aberrant glycosylation changes on many glycoproteins are often observed, and much experimental evidence has revealed that these structural changes are related to tumor malignancy (*8*). Moreover, tumor-associated alterations of cell surface glycosylation play a critical role in metastasis of carcinoma cells by altering tumor cell adhesion or motility in a manner that either promotes or inhibits invasion and metastasis, and numerous experimental studies have revealed that these structural changes are related to cancer progression and metastasis (*8*). In addition, cancer is correlated to aberrant expression of sialic acid (*9*−*11*). For example, cancer cells or tissues display enhanced expression of α-2,6-sialoglycans compared with normal cells or tissues, and the level of α-2,6 sialoglycan expression in colorectal tumor cells has been associated with metastatic capacity (*10*, *12*). Moreover, decreased

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the levels of α-2,3-sialoglycan are correlated with inhibition of migration and metastatic potential of B16F10 cells (*10*). Understanding how alteration of cell surface-binding motif in tumor cells will be one of the important goals in cancer progression and metastasis (*8*).

1-Deoxynojirimycin (1-DNJ), which is rich in mulberry leaves (*13*), is an analogue of glucose that contains an NH group substituting for the oxygen atom of the pyranose ring (also termed iminosugar or azasugars). 1-DNJ (*14*) and other iminosugars, such as castanospermine (*7*, *15*), swainsonine (*15*, *16*), and 1,6-epi-cyclophellitol (*17*), have been shown to inhibit experimental animal metastasis. Swainsonine has been reported to show evidence of clinical efficacy in a phase I clinical trial (*18*). In addition, a 1-DNJ analogue, *N*-butyldeoxygalactonojirimycin (NB-DNJ), has been found to reduce ganglioside metabolism in murine melanoma MEB4 tumor cells and to delay tumor onset in mice injected with MEB4 cells (*19*).

Melanoma-related metastasis is one of the major causes of cancer death in the United States. 1-DNJ has been shown to inhibit pulmonary colonization of mouse B16 melanoma by approximately 80% in an in vivo model (*14*). 1-DNJ may rapidly enter the cell, because iminosugars have been shown to enter HL60 cells within 1 min (*20*). However, it is unclear whether 1-DNJ inhibits the metastasis of the highly metastatic B16F10 melanoma cells directly and how this iminosugar exerts its antimetastatic effects. In this study, we examined our hypothesis that 1-DNJ as an oligosaccharide-processing inhibitor may inhibit the metastasis of B16F10 melanoma cells and we determined the possible mechanisms of such an action of 1-DNJ.

MATERIALS AND METHODS

Materials. The purified 1-DNJ (>98%) was purchased from Wako Pure Chemical Industries, Ltd. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Dulbecco's phosphate-buffered saline (DPBS), and penicillin/streptomycin were purchased from GIBCO. The lectins concanavalin A (ConA) and *Phaseolus vulgaris* leucoagglutinin (PHA-L) were prepared from their natural sources and labeled with fluorescein isothiocyanate (FITC) (Sigma, St. Louis, MO) in this laboratory. The following lectins were purchased from EY laboratories (San Mateo, CA) as FITC-conjugated: Maackia amurensis agglutinin (MAA) and *Sambucus nigra* lectin (SNA).

Cell Proliferation Assay. Cell proliferation was measured using the Promega Cell-Titer96 aqueous 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS), assay. The number of viable cells that remained attached to the bottom of the plate was measured by the conversion of MTS (Promega Cell-Titer96) to the aqueous soluble formazan accomplished by dehydrogenase enzymes found in metabolic active cells. The amount of formazan product detected at 492 nm by microplate multimode detector is directly proportional to the number of living cells in the culture.

Cell-Matrix Adhesion Assay. B16F10 cells (1×10^5 cells/well) were incubated with different concentrations of 1-DNJ (0, 2.0, 10, 20, 50, and 100 μg/mL) for 24 h. Ninety-six-well tissue culture plates were coated with 10 μg/well Matrigel overnight at 4 °C and were washed three times with PBS. B16F10 cells were resuspended in DMEM containing 0.1% BSA and incubated for 2 h. Nonadherent cells were removed by washing three times with PBS. Then the cells that remained attached to the bottom of the plate were measured by the MTS assay, and the absorbance was measured at 492 nm by microplate multimode detector.

Cell Migration and Invasion Assays. B16F10 cells were treated with 1-DNJ (0, 2.0, 10, 20, 50, and 100 μg/mL 1-DNJ in 50 μL of serum-free DMEM medium) as indicated. Cell migration was assessed using a QCM 24-well fluorometric cell migration assay kit (Chemicon) following the manufacturer's instructions. Briefly, B16F10 cells were incubated for 6 h at 37 °C that migrated through an 8 μm pore size polycarbonate membrane, then incubated with "cell stain solution", and subsequently extracted and detected on a multifunctional microplate reader using a 480/520 nm filter. Cell invasion was assessed using the Chemicon cell invasion assay kit consisting of a 24-well tissue plate with culture inserts (transmembrane chamber), which contain an 8 μm pore size polycarbonate membrane over which a thin layer of ECMatrix is dried. The extracellular matrix layer occludes the membrane pores, blocking noninvasive cells from migrating through. The plate was then incubated for 24 h at 37 °C to allow invading cells to migrate through the extracellular matrix layer and to cling to the bottom of the polycarbonate membrane. The insert membrane with invaded cells on the bottom was placed in the wells with cell stain/dissociation solution after incubation and reincubated for 30 min at 37 °C. Fluorescence was measured with a multifunctional microplate reader at 480/520 nm. Each experiment was repeated three times, and data represent the mean ± SD of three determinations.

Table 1. Primer Sequences Used for Real-Time Quantitative Polymerase Chain Reaction[a]

	5′−3′ sequence
MMP-2	
forward	5′-CTG GAA TGC CAT CCC TGA TAA-3′
backward	5′-CAA ACT TCA CGC TCT TGA GAC TTT-3′
MMP-9	
forward	5′-CAG GAG TCT GGA TAA GTT GGG TCT A-3′
backward	5′-ACG CCC CTT GCT GAA CAG-3′
TIMP-1	
forward	5′-CAT GGA AAG CCT CTG TGG AT-3′
backward	5′-CTC AGA GTA CGC CAG GGA AC-3′
TIMP-2	
forward	5′-GCA TCA CCC AGA AGA AGA GC-3′
backward	5′-GTC CAT CCA GAG GCA CTC AT-3′
β-actin	
forward	5′-GGC TGT ATT CCC CTC CAT CG-3′
backward	5′-CCA GTT GGT AAC AAT GCC ATG T-3′

[a] MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitors of metalloproteinase-1; TIMP-2, tissue inhibitors of metalloproteinase-2.

Gelatin Zymographic Assays of MMP-2 and MMP-9. Culture medium was electrophoresed on a 10% SDS-PAGE gel impregnated with 0.1% gelatin (w/v). Cells were washed two times for 30 min in 50 mM Tris-HCl (pH 7.5) plus 2.5% Triton X-100 followed by an overnight incubation at 37 °C in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.05% NaN_3. Gels were stained with Coomassie Brilliant Blue R-350 and then destained in acetic acid/methanol and dried. Quantification of the banding pattern was performed by densitometry. Each sample lane was scanned and presented as optical density (OD) in percent of control sample.

mRNA Expression of MMP-2/9 and TIMP-1/2 Determined by Quantitative Real-Time Polymerase Chain Reaction (PCR). Total RNA was isolated from 5×10^6 B16F10 cells using the RNeasy Mini kit (Qiagen). cDNA was obtained from total RNA (2 μg) by reverse transcription using reverse transcriptase (Invitrogen Life Technologies) according to the manufacturer's protocol. Primers for the quantitative detection of target mRNAs were designed (**Table 1**) by means of Primer Express computer software (Applied Biosystems). Real-time PCR to amplify the TIMP-1 and TIMP-2 genes was performed on an ABI StepOne Plus machine (Applied Biosystems) using a SYBR Green kit (Invitrogen Life Technologies). After an initial incubation step at 95 °C for 15 min, 40 PCR cycles were performed. The cycling conditions consisted of a denaturation step at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min. To confirm amplification specificity, the PCR products were subjected to a melting curve analysis. β-Actin was used as a relative and endogenous gene reference to correct difference for total RNA added to reaction and to compensate for different levels of inhibition during reverse transcription of RNA and during PCR. Relative gene expression of the genes of interest was expressed as a ratio of the expression level of the gene of interest to that of β-actin.

Fluorescein Labeling of Lectins and Flow Cytometric Analysis of Cell Surface. B16F10 cells were stained with fluorescin-labeled plant lectins and analyzed by flow cytometry. Cultured cells were treated with 1-DNJ for 24 h in a 6-well plate. B16F10 cells were then harvested and washed with PBS. Approximately 5×10^5 cells were labeled with 200 μL of lectin-specific sugars, for example, ConA and PHA-L, 2 μg/mL, or SNA

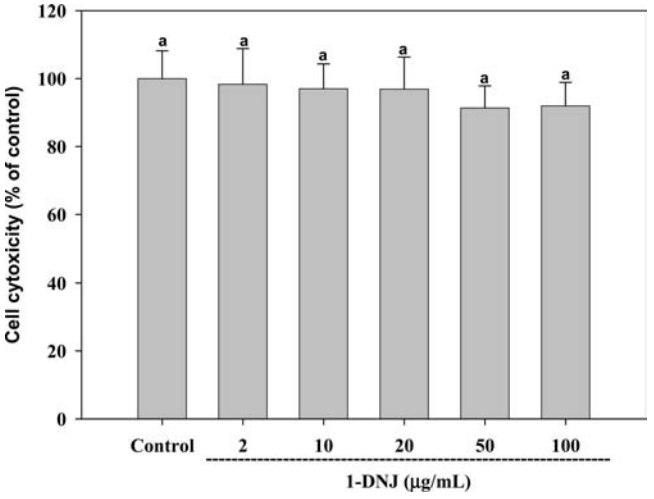

Figure 1. Effects of 1-deoxynojirimycin (1-DNJ) on cytotoxicity of B16F10 cells. Cells were incubated with 1-DNJ for 24 h. Data (mean ± SD, $n = 3$) are percentages of the control; means without a common letter differ, $P < 0.05$.

and MAA, 2 μg/mL, for 1 h at 4 °C, and then cells were sorted according to the intensity of the fluorescence they emitted. ConA and PHA-L can recognize α-mannose residues as well as complex branched-chain oligosaccharides that contain tri- and tetra-antennary α-linked *N*-acetylgalactosaminyl (GalNAc) on mannose residues of asparagine-linked oligosaccharides, respectively. Cells were washed twice with PBS, and fluorescence histogram profiles were determined using flow cytometry (Becton-Dickon Co.) for fluorescence analysis. Fluorescence-activated cell spectra (FACS) were drawn automatically, and the curve or its peak indicated the fluorescence intensity.

Statistical Analyses. Data (mean ± SD) are analyzed by using one-way ANOVA followed by least significant difference (LSD) test for multiple comparisons of group means. A P value < 0.05 is considered to be statistically significant.

RESULTS

Cytotoxicity of 1-DNJ to B16F10 Cells. Microscopic examination of B16F10 cell morphology and MTS assay revealed no significant difference between control and 1-DNJ-treated cells at least up to 100 μg/mL (615 μM) (**Figure 1**). Thus, we used 0−100 μg of 1-DNJ/mL in all subsequent experiments.

Inhibition of Cell-Matrix Adhesion, Cell Migration, and Invasion by 1-DNJ. 1-DNJ significantly suppressed the invasion (**Figure 2A**) and adhesion (**Figure 2C**) of B16F10 cells, and the effects were dose-dependent up to 20 μg/mL (123 μM). An EC_{50} of approximately 15 μg/mL (92 μM) was obtained from **Figure 2A**,**C** for 1-DNJ in the inhibition of both invasion and adhesion of B16F10 cells. 1-DNJ also inhibited cell migration, but the effect was much weaker than those on adhesion and invasion, and no EC_{50} value could be obtained (**Figure 2B**).

Inhibition of Matrix Metalloproteinase (MMP-2 and MMP-9) Activities by 1-DNJ. Incubation of B16F10 cells with 1-DNJ for 24 h resulted in relatively mild but concentration-dependent inhibition of MMP-2, and the effect was significant only at 50 μg of 1-DNJ/mL (or 308 μM) (**Figure 3**). 1-DNJ also caused mild and concentration-dependent inhibition of MMP-9, and the effect was significant (by 20%, $P < 0.05$) only at 100 μg DNJ/mL (or 615 μM).

Expression of Matrix Metalloproteinase (MMP-2 and MMP-9) mRNA by 1-DNJ. Incubation of B16F10 cells with 1-DNJ for 16 h markedly decreased the MMP-2 mRNA expression, with an inhibition of 60% ($P < 0.05$) at 2 μg of 1-DNJ/mL (12 μM), but no further decrease in the expression was seen at higher

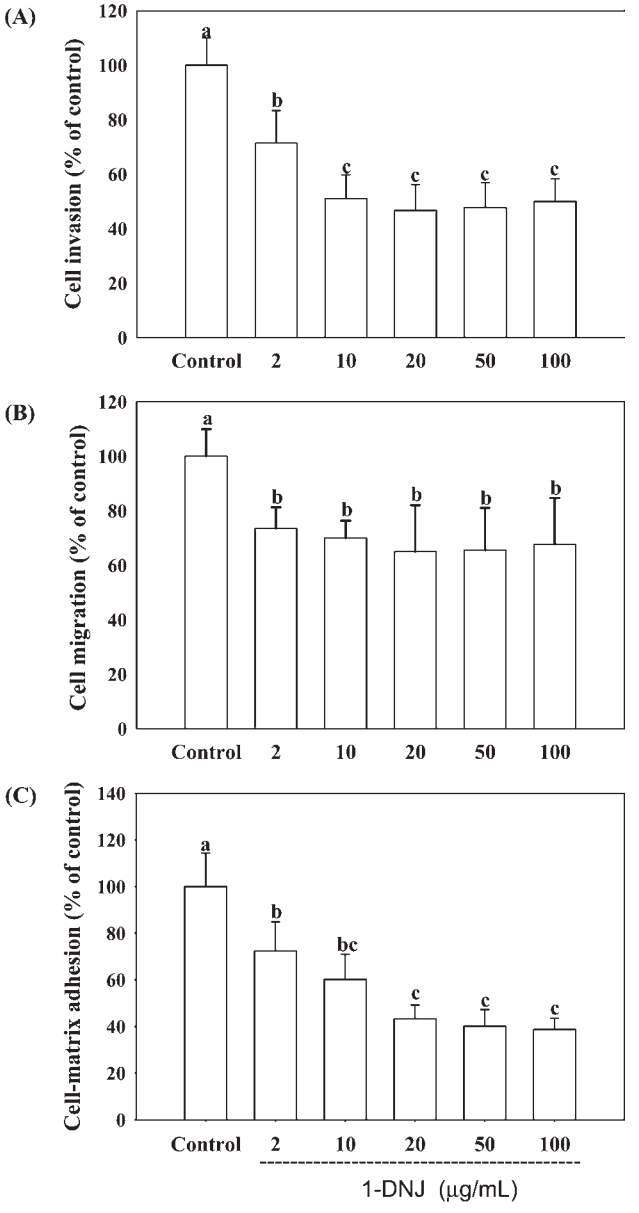

Figure 2. Effects of 1-deoxynojirimycin (1-DNJ) on invasion (**A**), migration (**B**), and cell-matrix adhesion (**C**) of B16F10 cells. Cells were incubated with 1-DNJ for 24 h. Data (mean ± SD, $n = 3$) are percentages of the control; means without a common letter differ, $P < 0.05$.

1-DNJ concentrations (**Figure 4**). 1-DNJ also significantly decreased the MMP-9 mRNA expression (with 28% decrease, $P < 0.05$, at 20 μg/mL or 123 μM 1-DNJ), and the effects were dose-dependent up to 100 μg/mL (615 μM).

Expression of Tissue Inhibitors of Metalloproteinase (TIMPs) mRNA by 1-DNJ. Incubation of B16F10 cells with 1-DNJ for 16 h did not significantly affect TIMP-1 mRNA expression in B16F10 cells (**Figure 5**). In contrast, 1-DNJ markedly increased TIMP-2 mRNA expression, and the increase became statistically significant, when the 1-DNJ concentration reached 2 μg/mL or 12.3 μM (31%, $P < 0.05$). In fact, 1-DNJ increased the expression of TIMP-2 mRNA by 3.4- and 8.2-fold at 50 and 100 μg/mL, respectively, as compared to the expression level of control.

1-DNJ Alters the Glycosylation and Sialylation of Cell Surface. To modify the glycoprotein oligosaccharide motif of B16F10 cell membrane, subconfluent cultures were incubated with 1-DNJ for 24 h. This time is equivalent to the doubling time of B16F10 and allows substantial turnover of cellular glycoproteins. 1-DNJ

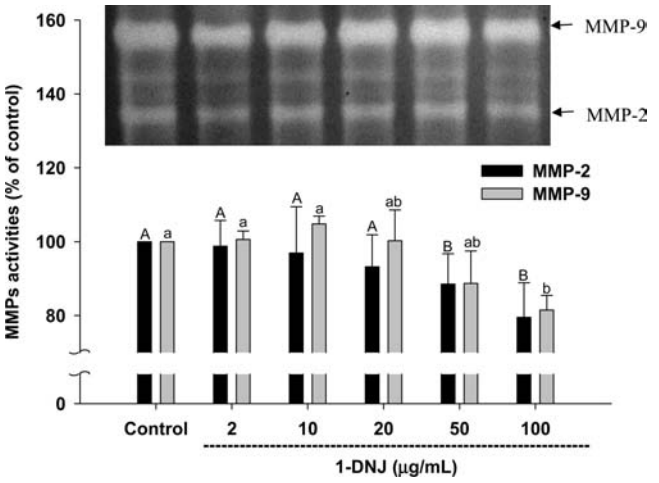

Figure 3. Effects of 1-deoxynojirimycin (1-DNJ) on MMP-2 and MMP-9 activities in B16F10 cells. Cells were incubated with 1-DNJ for 24 h, and the activity was measured by gelatin zymography. Data (mean ± SD, $n = 3$) are expressed as percent of control; means without a common letter differ, $P < 0.05$.

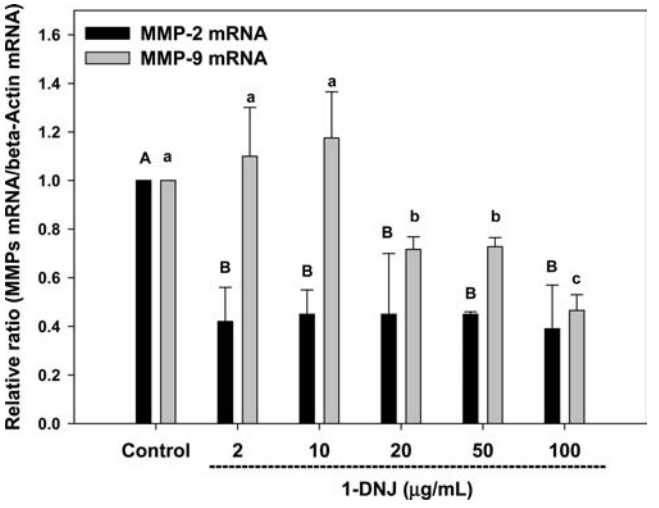

Figure 4. Effects of 1-deoxynojirimycin (1-DNJ) on MMP-2 and MMP-9 mRNA expression in B16F10 cells. Cells were incubated with 1-DNJ for 16 h, and the expression was measured by quantitative real-time PCR. Data (mean ± SD, $n = 3$) are expressed as fold decrease over control; means without a common letter differ, $P < 0.05$.

elevated the level of α-mannose at the cell surface, which is recognized by FITC-labeled ConA, with 49% increase ($P < 0.05$) at 100 μg of 1-DNJ/mL (615 μM) (**Figure 6A**). By contrast, 1-DNJ decreased the levels of tri- and tetra-antennary β-linked GalNAc that are recognized by FITC-labeled PHA-L, with 21% inhibition ($P < 0.05$) at 100 μg of 1-DNJ/mL (615 μM) (**Figure 6B**).

1-DNJ decreased the level of sialoglycan containing α-2,3-linked sialic acid residues, which are the MAA-binding motif of cell membrane (**Figure 7A**), with 30% inhibition ($P < 0.05$) at 100 μg of 1-DNJ/mL (615 μM). Similarly, 1-DNJ decreased the level of sialoglycan containing α-2,6-linked sialic acid residues, which are the SNA-binding motif of cell membrane (**Figure 7B**), with 32% inhibition ($P < 0.05$) at 100 μg of 1-DNJ/mL (615 μM).

DISCUSSION

1-DNJ has been reported to inhibit metastatic activity in an animal model (*14*), but the mechanisms underlying such actions are not clear. In the present study, we show that 1-DNJ inhibited

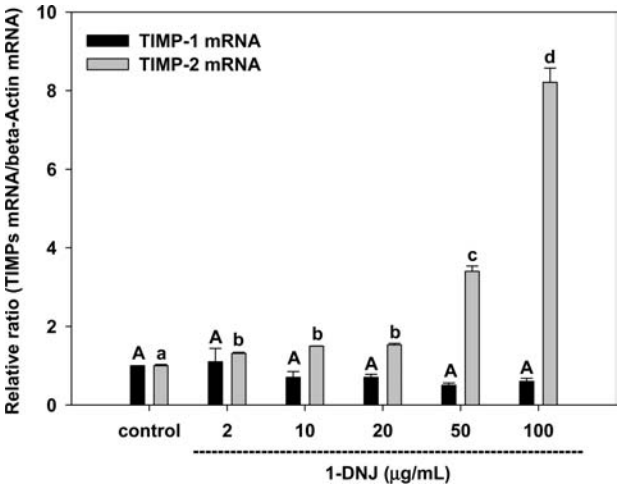

Figure 5. Effects of 1-deoxynojirimycin (1-DNJ) on TIMP-2 mRNA expression in B16F10 cells. Cells were incubated with 1-DNJ for 16 h, and the expression was measured by quantitative real-time PCR. Data (mean ± SD, $n = 3$) are expressed as fold increase over control; means without a common letter differ, $P < 0.05$.

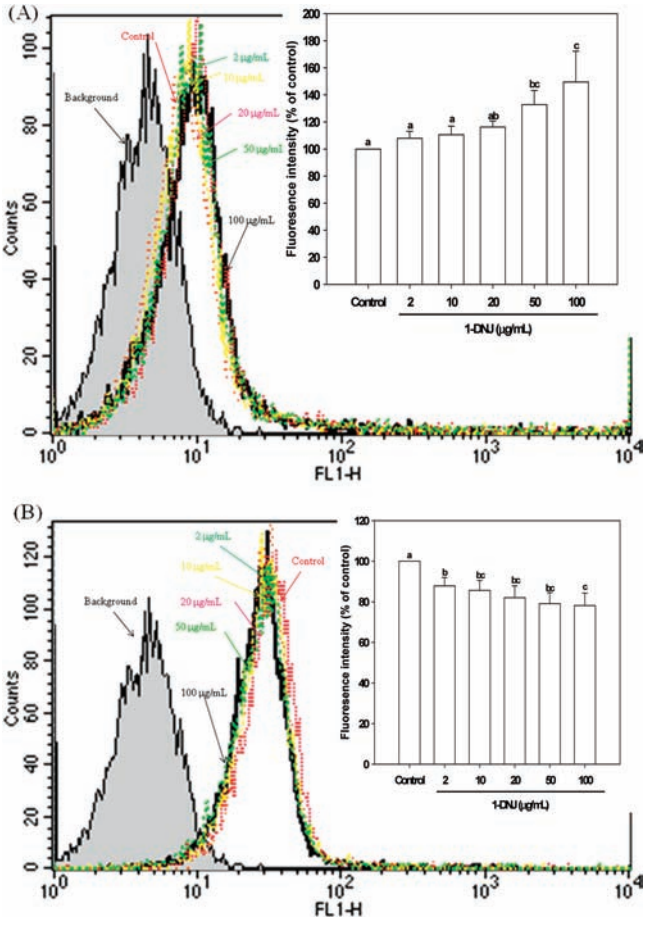

Figure 6. Effects of 1-deoxynojirimycin (1-DNJ) on glycosylation of B16F10 cell surface. Cells were incubated with 1-DNJ for 24 h. The level of terminal α-mannose at the cell surface was recognized by FITC-labeled ConA (**A**), and the level of β-1,6-branched tri- and tetra-antennary oligosaccharides at the cell surface was recognized by FITC-labeled PHA-L (**B**). Data (mean ± SD, $n = 3$) are percentage fluorescence intensity of the control; means without a common letter differ, $P < 0.05$.

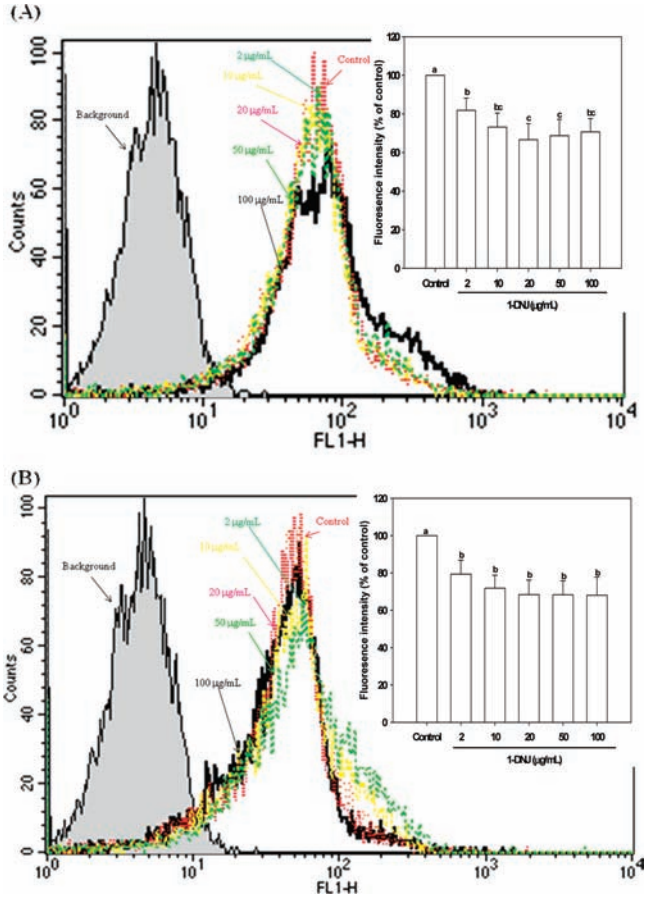

Figure 7. Effects of 1-deoxynojirimycin (1-DNJ) on sialylation of B16F10 cell surface. Cells were incubated with 1-DNJ for 24 h. The level of α-2,3-sialoglycan at the cell surface was recognized by FITC-labeled MAA (**A**), and the level of α-2,6-sialoglycan at the cell surface was recognized by FITC-labeled SNA (**B**). Data (mean ± SD, $n = 3$) are percentage fluorescence intensity of the control; means without a common letter differ, $P < 0.05$.

the metastasis of melanoma B16F10 cells, as evidenced by significantly decreased cell adhesion, migration, and invasion and reduced invasion and cell-matrix adhesion. Mechanistically, we show that 1-DNJ suppressed the activities and expression of MMP-2 and MMP-9 activities in B16F10 cells. In contrast, 1-DNJ effectively enhanced the expression of TIMP-2 mRNA. We also demonstrated that 1-DNJ significantly decreased glycosylation and/or sialylation on B16F10 melanoma cell surface but increased the levels of α-mannose.

A probable mechanism underlying the antimetastatic action of 1-DNJ against B16F10 melanoma cells is the inhibition of expression and activities of MMP-2 and MMP-9, as we found that 1-DNJ inhibited both MMP-2 and MMP-9 mRNA expression, with MMP-2 being much more strongly inhibited by 1-DNJ. It should be noted that 1-DNJ inhibited the activities of MMP-2 and MMP-9 to similar extents, whereas this iminosugar was more effective in inhibiting the mRNA expression of MMP-2 than that of MMP-9. A possible explanation is that most MMPs respond to stimuli at the mRNA level with delayed kinetics over several hours and require ongoing translation (*21*). In addition, 1-DNJ may regulate MMP activities at different levels, such as gene transcription, post-transcriptional mRNA stabilization, translational efficiency, enzyme compartmentalization, secretion, and zymogen activation (*22*). Because 1-DNJ markedly enhanced the mRNA expression of TIMP-2, there was a strong imbalance in the ratio of MMP-2 and TIMP-2, which could lead to effective inhibition of the metastasis of B16F10 cells. Indeed, it has been shown that small shifts in the balance between MMPs and TIMPs significantly influence the invasive phenotype of cancer cells (*23*) and that overexpression of TIMPs in B16F10 melanoma cells reduces their metastatic potential (*24*, *25*). In particular, the balance in the ratio of MMP-2 and TIMP-2 is considered tobe important in the regulation of tumor metastasis, angiogenesis, and growth.

Another possible mechanism underlying the antimetastatic action of 1-DNJ is through its inhibition of tumor cell-extracellular matrix adhesion, as we found that 1-DNJ strongly and dose-dependently inhibited cell-matrix adhesion in B16F10 cells. In the metastatic cascade, cell oligosaccharides could be in mediation of cell-matrix interactions because rapid binding of the cells to exposed macromolecules on the extracellular matrix surface is critical for the arrest of metastatic cells (*26*). In addition, changes of cell-surface oligosaccharides by 1-DNJ may interfere with the adhesiveness of B16F10 cells, as studies have suggested that abnormal glycosylation and sialylation on tumor cell surface are associated with metastasis (*8*, *10*, *27*). In the present study, we found that 1-DNJ significantly and dose-dependently increased the levels of α-mannose, whereas 1-DNJ significantly and dose-dependently decreased the levels of β-1,6-branched tri- and tetra-antennary oligosaccharides, α-2,3- and α-2,6-sialoglycans, in cell membranes. Indeed, studies have demonstrated a positive correlation between the surface expression of β-1,6-branched oligosaccharides and metastasis (*26*). Furthermore, 1-DNJ has been shown to inhibit glucosidase activity and oligosaccharide processing of glycoprotein of cell membrane (*28*). Therefore, our findings that 1-DNJ dose-dependently decreased the enzyme activity suggest that the change of cell-surface oligosaccharides by 1-DNJ may, at least in part, be due to its inhibition of α-glucosidase activity.

In this study, we observed an EC_{50} of 15 μg/mL (or 92 μM) for 1-DNJ in reducing invasion and cell-matrix adhesion of B16F10 melanoma cells. Such a level of 1-DNJ is relatively high but appears to be achievable by feeding high doses of 1-DNJ to rats. Indeed, Nakagawa et al. (*13*) have shown that the plasma level of 1-DNJ reaches 15 μg/mL (92 μM) in rats 30 min after a single oral administration of 1-DNJ (110 mg/kg of body weight), although the plasma 1-DNJ level decreases rapidly thereafter. Because the ingested 1-DNJ is rapidly eliminated in an intact form in the rat, no side effects are found (*13*). It is important to note that the antimetastatic effect of 1-DNJ was not a result of cytotoxicity, as we found no cytotoxicity for 1-DNJ at concentrations up to 100 μg/mL (615 μM). 1-DNJ also does not inhibit the proliferation of bovine aorta endothelial cells at concentrations up to 1 mM (*29*).

In conclusion, the present study demonstrates that 1-DNJ reduces B16F10 cell metastatic capability, and this effect of 1-DNJ is, at least in part, attributed to the suppression of the activity and expression of MMP-2/-9 and to the imbalance of MMP-2 and TIMP-2 as well as to the alterations of the cell surface-binding motif. These findings suggest that 1-DNJ may be useful as an adjuvant of antimetastatic agents such as cisplatin.

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