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5′-Nitro-Indirubinoxime inhibits SGT cell invasion by targeting β1 integrin/Akt signaling pathway

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β1 integrin/Akt 신호전달을 타겟으로 하는
5′-Nitro-Indirubinoxime의 타액선암 세포 침습 억제 효과

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이 논문을 이학 석사학위신청 논문으로 제출함

2009년 4월 일

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Abstract

5′-Nitro-Indirubinoxime inhibits SGT cell invasion by targeting β1 integrin/Akt signaling pathway

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Background: 5'-nitro-indirubinoxime (5'-NIO) is a derivative of the bis-indole indirubin, an active compound of Danggui Longhui Wan that exhibits anti-leukemic activities. Previously, we have shown that 5'-NIO has potent anti-tumor effect in various human cancer cells. But the potential anti-invasive effect of 5'-NIO in salivary gland tumor has not been studied yet. The goal of this study is to evaluate the anti-invasive action of 5'-NIO in salivary gland adenocarcinoma SGT cells.

Methods: The MTT assay was used to determine the viability of SGT cells after treatment with 5'-NIO. The wound-scratch, migration and invasion assays were applied to determine the effect of 5'-NIO on the migration capacity and invasiveness of SGT cells. Western blot and RT-PCR were performed to evaluate the impacts of 5'-NIO on the expression of MMP-2/-9 and its upstream signaling molecules. The secretions of MMP-2/-9 in culture-conditioned medium were assayed by gelatin zymography and adhesion assay was performed using type I collagen.

Result: 5'-NIO exhibited a dose-dependent inhibitory effect on the invasion

and migration of SGT cells. 5'-NIO decreased β1 integrin expression and phosphorylation of FAK, Akt, Erk, and downregulated the expression of MMP-2/-9 in SGT cells. Furthermore, 5'-NIO suppressed MMP-2/-9 mRNA expression and activity.

Conclusion: Taken together, these results suggest that 5'-NIO could effectively inhibit the invasion and migration of human SGT cells by downregulating the expression of β1 integrin and MMP-2/-9 and phosphorylation of FAK, Akt, and Erk.

국문초록

β1 integrin/Akt 신호전달을 타겟으로 하는 5′-Nitro-Indirubinoxime의 타액선암 세포 침습 억제 효과

연구배경: 5‘-Nitro-Indirubinoxime은 bis-indole indirubin의 유도체로서, Danggui Longhui Wan의 구성 성분이다. 5‘-NIO는 여러 종양세포에서 강력한 항암효과가 있다. 그러나 5‘-NIO의 항침습 효과는 연구된 바 없다. 이 논문은 타액선암 세포에서 5’-NIO의 항침습 효과를 평가하고자 한다.

실험방법: 타액선암 세포의 생존율을 보기 위해 5‘-NIO 처리 후 MTT assay를 하였고, 세포의 침습과 전이능을 확인하기 위해 wound-scratch, migration과 invasion assay를 시행하였다. MMP-2와 MMP-9, 그리고 이들의 상위신호분자들의 발현을 western blot과 RT-PCR을 하여 확인하였다. 또한, gelatin zymography를 하여 MMP-2와 MMP-9의 분비를 확인하고, β1 integrin의 ligand 중 하나인 type I collagen을 이용하여 adhesion assay를 시행하였다.

실험결과: 5’-NIO는 타액선암 세포에서 농도 의존적으로 세포이동과 침습능 억제효과를 나타내었고, β1 integrin의 발현, FAK, Akt, Erk의 인산화와 MMP-2/-9의 발현이 감소됨을 볼 수 있었다. 또한, MMP-2/-9의 mRNA 발현과 분비가 억제됨을 확인하였고, type I collagen에서 5’-NIO의 농도 의존적으로 타액선암 세포의 부착능력이 감소됨을 규명하였다.

결론: 이러한 결과는 5‘-NIO가 FAK, Akt와 Erk의 인산화, β1 integrin, MMP-2/-9의 발현을 조절함으로 타액선암 세포의 이동과 침습능 억제에 효과적임을 확인하여 타액선암 치료의 또 다른 가능성을 확인하였다.

Ⅰ. Introduction

Indirubin is an active compound of Danggui Longhui Wan, which is used to treat chronic diseases in traditional Chinese medicine (1-3). Recent several studies have shown that indirubin derivatives have anti-tumor effect on human cancer cells through the inhibition of cyclin-dependent kinases (CDKs) and suppress the cell growth by arresting the cell cycle (1, 4-8). Recently, we synthesized a novel indirubin derivative, 5'-Nitro-Indirubinoxime (5'-NIO), and it has shown the most potent antitumor activity in RK3E-ras cell using *in vitro* and *in vivo* studies (2). Moreover, we have demonstrated the action of 5'-NIO-induced antitumor activity in oral squamous carcinoma KB cells. 5'-NIO inhibits cell proliferation via the inhibition of the G1/S and G2/M phase regulatory protein (6).

Salivary gland cancers account for approximately 3-6% of all head and neck tumors in adults (9-11). Salivary gland tumor contains cancers of different histological characteristics and biological behavior (10). Several studies have emphasized the importance of oncogenes in the development of salivary gland cancers (10, 12-15). Although salivary gland cancer is relatively rare and its etiology is unknown, the involvement of environmental or genetic factors has been suggested. Malignant salivary gland cancers have not been associated with smoking and excess of alcohol intake compare with most other head and neck cancers (11, 16). The functions of chemotherapy in the management of salivary gland cancers remain unsolved (10). Dodd et al. reported response rates of salivary gland cancers to chemotherapy are inconsistent, varied and generally poor. Malignant salivary gland tumors are highly aggressive neoplasms that readily invade adjacent tissues and metastasize to distant organs at early stages of the disease. Therefore, inhibition of tumor invasion and migration in salivary gland cancer therapy is very important.

The principal mechanisms of cancer cell invasion and migration entail a complex series of events. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which is degrading extracellular matrix (ECM) and basement

membrane (17-19). Among the MMPs, MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) are overexpressed in various malignant tumors and play critical roles in tumor invasion and migration (20-22).

As well as MMPs, the integrin members are also known to mediate invasion and migration. Integrin receptors are playing critical roles in cell-to-cell contacts, cell adhesion and migration, and major receptor for cell adhesion and extracellular matrix (ECM) (23). Among the integrins, β1 integrin has been well known to mediate cell adhesion and migration, and expression of MMP-2/-9. After ligand stimulation or integrin engagement, focal adhesion kinase (FAK) becomes phosphorylated, which leads the phosphorylation of down regulator such as Akt and Erk. Subsequently, activation of these signaling molecules leads to increase of MMPs production, promoting invasion of migration of tumor cells (24-28).

As mentioned above, we has been shown that antitumor effect of 5'-NIO *in vitro* and *in vivo* (2, 3). However, the anti-invasive effects of 5'-NIO in salivary gland tumor has not been studied yet. Therefore, in this study, we investigated the inhibitory effects of 5'-NIO on salivary gland adenocarcinoma SGT cell invasion and migration by targeting β1 integrin/Akt signaling pathway.

Ⅱ. Materials and methods

1) Cell culture

SGT human salivary gland adenocarcinoma cells (29) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (GIBCO), 100 μg/ml streptomycin and 100 units/ml penicillin at 37℃ in a 5% CO_2 humidified incubator. Cells were treated with different concentrations of 5′-NIO for 24 h.

2) Cell proliferation assay

Cell viability was determined using the MTT assay. The cells were seeded at 3×10^5 cells/ml into the 12 well plates. Cells were treated with 5′-NIO (dissolved in 0.1% DMSO) for 24 h. Cells were washed twice with ice-cold PBS, and 0.5 ml of cell culture medium and 50 μl of 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenylte-trazolium bromide reagent (5 mg/ml in PBS) were added. After incubation 3 h at 37℃ the media was removed and 250 μl of acid-isopropanol (0.04 mol/L HCL in isopropanol) were added. The optical density (OD) value of the dissolved solute was then measured by a Microplate Autoreader ELISA (Bio-Tek Instruments Inc., Winooski, VT) at 570 nm wavelength.

3) Matrigel invasion assay

Cell invasion assay was performed using a Matrigel invasion assay kit (BD Biosciences) by measuring the according to the manufacturer's instructions. Briefly, serum free media supplemented with 5′-NIO was added to the well. Cells were seeded in the insert of the transwell plate and incubated for 22 h at 37℃ The cells that invaded to the lower surface of the membrane were fixed with methanol and stained with hematoxylin for 5 min. At × 200 magnification, five areas from each sample was randomly selected and captured using an Olympus BX41 inverted microscope and invasion cells were counted as previously described (30).

4) Wound-scratch assay

After the cells were allowed to attach and reach confluence in 60 mm culture dish, a scratch (3 mm) was made through the culture dish. The cells were washed twice with PBS before their subsequent incubation with culture medium in the presence of 5′-NIO, and photographs were taken (× 100) in series at different time points. The percentage of wound closure was evaluated using the formula (size after treated reagent/ initial wound size) × 100. The experiments were performed in triplicate (31).

5) Cell migration assay

Cell migration assay was performed using a Chemotaxis Cell Migration Assay kit (CHEMICON) according to the manufacturer's instructions. The cells were collected by trypsinization and suspended in serum-free media at 2×10^5/ml. 5′-NIO was treated outside of the insert. The cells were allowed to migrate for 24 h at 37℃ in a humidified atmosphere containing 5% CO_2. The cells that migrated to the lower surface of the membrane were fixed with methanol and stained with hematoxylin for 5 min. Five (× 200) images from each sample was randomly selected and captured using an Olympus BX41 inverted microscope and migrated cells were counted (30). And migrated cells are lysed using the cell lysis buffer and which are determined with the CyQUANT GR Dye using fluorescence plate reader (Varioskan, Thermo Electron Co, Waltham, MA, USA) at 480/520 nm. As another experiment, 50 μl of type I collagen (20 μg/ml) was treated in 24-well plates for 3 h at 37℃. The cells were seeded in insert treated with 5′-NIO after treatment of 20 μg/ml type I collagen, and cell migration was evaluated using a Chemotaxis Cell Migration Assay kit.

6) Western blot analysis

SGT cells were seeded at 3×10^5 cells/ml into the 6-well plate and treated with 5′-NIO. The cells were lysed in lysis buffer (137 mM NaCl, 15 mM EGTA, 0.1 mM sodium orthovanadate, 15 mM $MgCl_2$, 0.1% Triton X-100, 25 mM MOPS, 100

μM phenylmethylsulfonyl fluoride and 20 μM leupeptin, adjusted to pH 7.2). Then protein concentrations were measured at 570 nm using Microplate Autoreader ELISA (Bio-Tek Instruments Inc., Winooski, VT). The total protein (40-50 μg per lane) was resolved by 7.5-15% SDS-PAGE and transferred onto PVDF membranes. After blocking in TBS (20 mmol/L Tris, 137 mmol/L NaCl, 1 g/L, pH 7.6) with 5% skim milk for 2 h at room temperature, the membranes were incubated with primary antibodies at 37℃ overnight. Antibodies of β1 integrin, FAK, pFAK, c-Myc, MMP-2 and MMP-9 were from Santa Cruz Biotechonology. And antibodies of Akt, pAkt, Erk and pErk were from Cell Signaling. The membranes were then washed three times with 0.05% tween 20-TBS (TBS-T), followed by incubation for 1 h with secondary antibodies (1:5000; Santa Cruz Biotechnology) at room temperature. Finally, the membranes were visualized using the West ZOL PLUS detection reagent in the LAS-1000.

7) RT-PCR

cDNA synthesis and reverse-transcription polymerase chain reaction (RT-PCR) for β1 integrin, MMP-2, MMP-9 and GAPDH mRNA were performed and the results were analyzed. cDNA was synthesized from 2 μg total RNA using an One Step RT-PCR kit. The PCR consisted of an initial denaturation at 94℃ for 2 min; 30 cycles of 94℃ for 15 sec, 55℃ for 30 sec and 72℃ for 1 min; a final extension at 72℃ for 10 min. The PCR products were separated on a 1.2% agarose gel, visualized, and photographed using a gel documentation system.

Table 1. Oligonucleotide sequences of PCR primers used in this study

Oligo	Prime	Sequence
β1 integrin	Forward	5′-ATGCCTACTTCTGCACGATGTG-3′
	Reverse	5′-GATGGCATCGAAACCACCTTCT-3′
MMP-2	Forward	5′-CTTCCAAGTCTGGAGCGATGT-3′
	Reverse	5′-TCTCCCAAGGTCCATAGCTCA-3′
MMP-9	Forward	5′-CCTGCAACGTGAACATCTTCG-3′
	Reverse	5′-GCCTGTGTACACCCACACCTG-3′
GAPDH	Forward	5′-CCAAGGTCATCCATGACAACT-3′
	Reverse	5′-GTCATACCAGGAAATGAGCTTGACA-3′

8) Gelatin zymography

MMP-2 and MMP-9 enzymatic activities were assayed by gelatin zymography. Supernatants from 5′-NIO-treated cultures were electrophoresed on an 8% SDS-PAGE containing gelatin. The gel was washed three times with renaturating buffer, followed by a brief rinsing in distilled water, and then incubated with development buffer at 37℃. After incubation, the gel was stained with Coomassie Brilliant Blue G250 (0.1% in 25% methanol and 10% acetic acid in water) and destained in the same solution in absence of the dye (17).

9) Cell adhesion assay

Type I collagen was diluted in PBS to stated concentrations, and 50 μl/well was added to 96-well plates and placed overnight at 4℃. After the coating, the wells were rinsed with PBS, and nonspecific binding sites were blocked with 1% BSA at 37℃ for 1 h. Different doses of 5'-NIO were applied to each well and the plates were incubated at 37℃ for 30 min. Subsequently, 5×10^4 cells were added in each well and allowed to adhere at 37℃ for 30 to 90 min. Nonadherent cells were rinsed off with PBS, and the remaining cells were fixed with 4% paraformaldehyde for 5 min. Cells were stained with 0.5% toluidine blue in 4% paraformaldehyde for 5 min and rinsed in water. Cells were solubilized with the addition of 100 μl of 1% SDS and quantified in a Microplate Autoreader ELISA (Bio-Tek Instruments Inc., Winooski, VT) at 595 nm (32).

10) Statistical analysis

The differences in mean values among different groups were tested, and the values were expressed as mean ± SD. All of the statistical calculations were carried out using Microsoft Excel. Values of $P<0.05$ were considered significant.

Ⅲ. Results

1. Effect of 5'-Nitro-Indirubinoxime on SGT cell viability

The cell viability of SGT cells was examined by MTT assay at the absence or presence of 5′-NIO. Compared with that of controls, the cell viability significantly decreased by 5′-NIO in a manner dependent on dose (Fig. 1). Treatment with 0.5 and 1 μM of 5′-NIO reduced cell viability by approximately 15%. Thus, these concentrations of 5'-NIO were determined to be used during subsequent experiments.

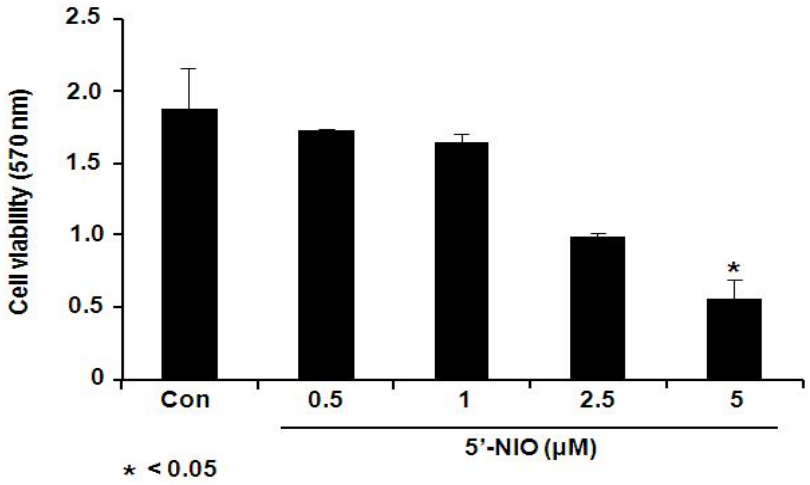


Fig. 1. Effect of 5′-Nitro-Indirubinoxime on SGT cell viability. SGT cells were incubated with different doses of 5′-NIO (0.5, 1, 2.5 and 5 μM) for 24 h. Cell viability rates were measured by MTT assay. 5′-NIO reduces cell viability in a dose-dependent manner in SGT cells.

2. Effect of 5'-NIO on SGT cell invasion and migration

Cell migration and invasion are of fundamental importance in tumor metastasis and angiogenesis (33, 34). To investigate whether 5'-NIO inhibits tumor invasion and

migration, matrigel invasion, wound scratch and migration assays were performed in 5'-NIO-treated SGT cells. Treatment with 0.5 μM 5'-NIO reduced the invasiveness of cells and 1 μM 5'-NIO significantly blocked tumor invasion (Fig. 2). In addition, 5'-NIO suppressed the migration of SGT cells across the wounded space in a time and dose dependant manner (Fig. 3A). This supported the results obtained from the migration assay (Fig. 3B, C and D). These results suggest that 5'-NIO may be effective for suppressing invasion and migration of salivary gland carcinoma.

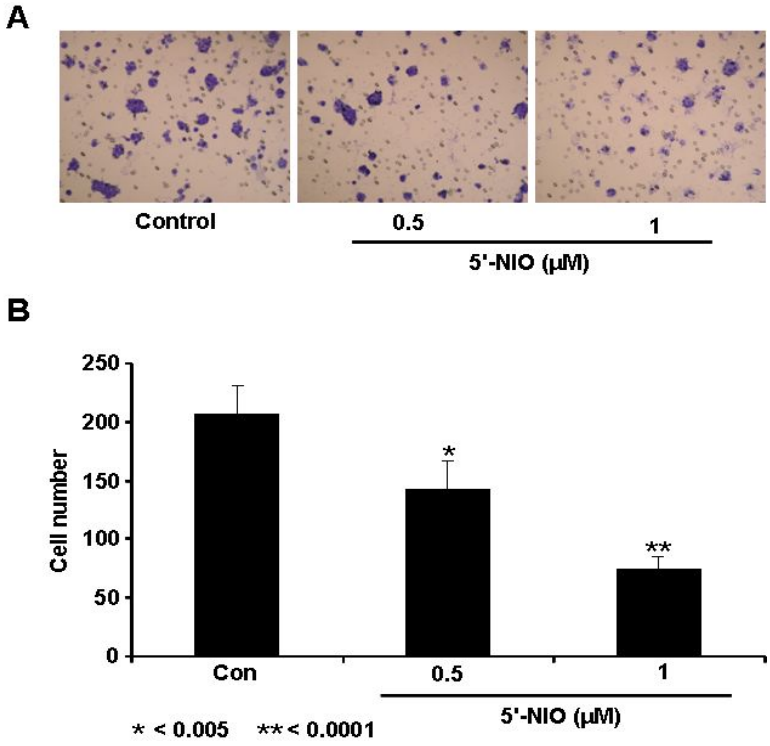
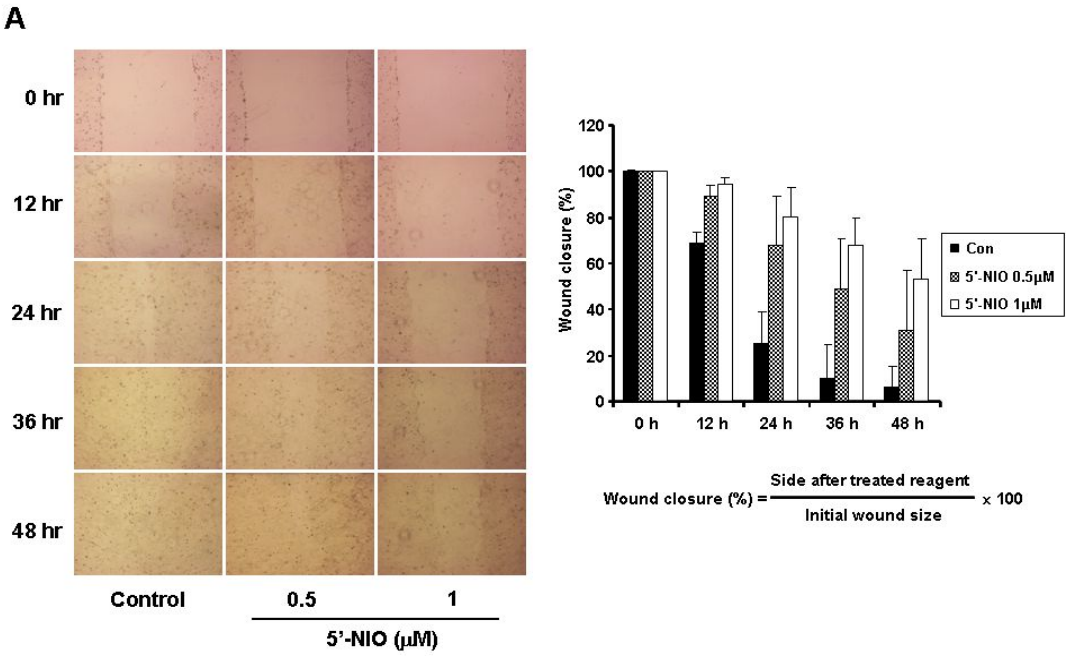
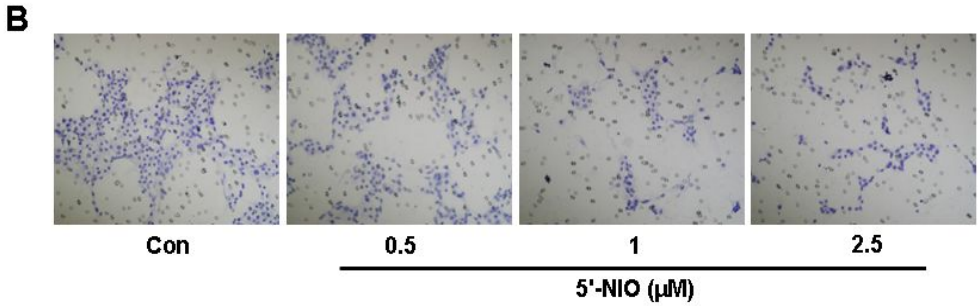


Fig. 2. Effect of 5′-NIO on SGT cell invasion. (A) The cells were cultured in the presence of various concentrations of 5′-NIO for 24 h within a matrigel invasion chamber. (× 200) (B) The invasiveness was quantified and is presented in the graph. 5′-NIO suppressed the invasiveness of SGT cells in a dose dependent manner.





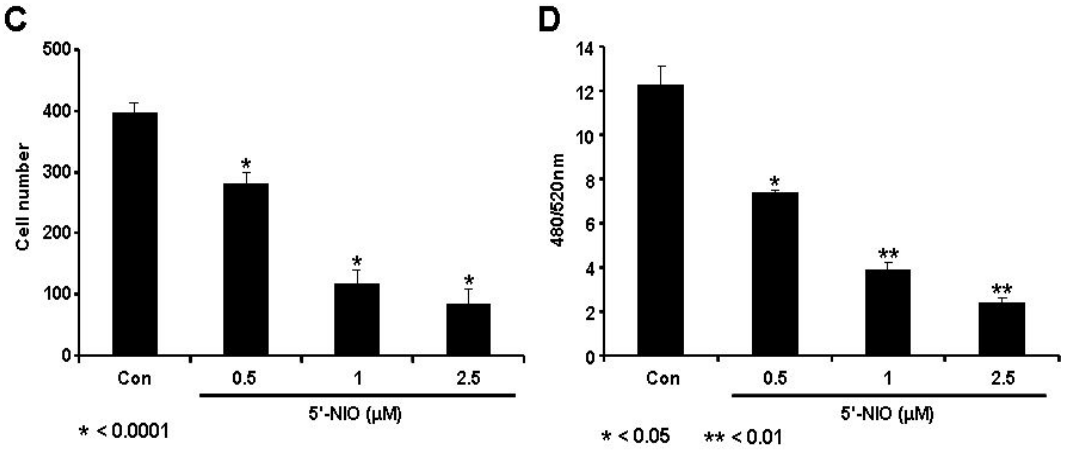


Fig. 3. Effect of 5′-NIO on SGT cell migration. (A) SGT cells were treated with 5′-NIO, and photographs were taken (× 100) in series at different time points. 5′-NIO blocked the migration of SGT cells across the wounded space in a time and dose dependant manner. (B∼D) The cells were cultured in the presence of various concentrations of 5′-NIO for 24 h within a migration chamber. (B) Microscopy images detected cells that migrated into the inner membrane. (× 200) (C) The cell migration was quantified by counting the number of cells that migrated into the inner membrane. Treatment with 5′-NIO significantly reduced the cell migration in a dose dependent manner. (D) Migrated cell number measured by relative fluorescence, and 5′-NIO inhibited cell migration in a dose dependent manner.

3. Effect of 5'-NIO on β1 integrin/Akt signaling pathway

Cell adhesion to extracellular matrix (ECM) proteins is mediated by integrins that play a significant role in tumor progression and metastasis (35). Recently, it has been reported that organ-specific sites of metastatic lesions are determined at least in part by β1 integrin-mediated adhesion to and invasion into the subendothelial extracellular matrix, and furthermore, different metastatic behaviors of tumors correlate with β1 integrin-mediated adhesive properties (36, 37). Treatment of 5'-NIO suppressed the production of cell surface β1 integrin (Fig. 4A). And phosphorylation of FAK, Akt, and Erk was also reduced by 5'-NIO in a dose-dependent manner (Fig. 4A). Moreover, 5'-NIO also inhibited the expression of c-Myc and MMP-2/-9 in a dose-dependent manner (Fig. 4A). To examine whether 5'-NIO affects the steady-state levels of β1 integrin, MMP-2, and MMP-9 mRNAs, RT-PCR was performed (Fig. 4B). The amount of mRNA expressions for β1 integrin, MMP-2, and MMP-9 were also reduced by 5'-NIO in a dose-dependent manner.

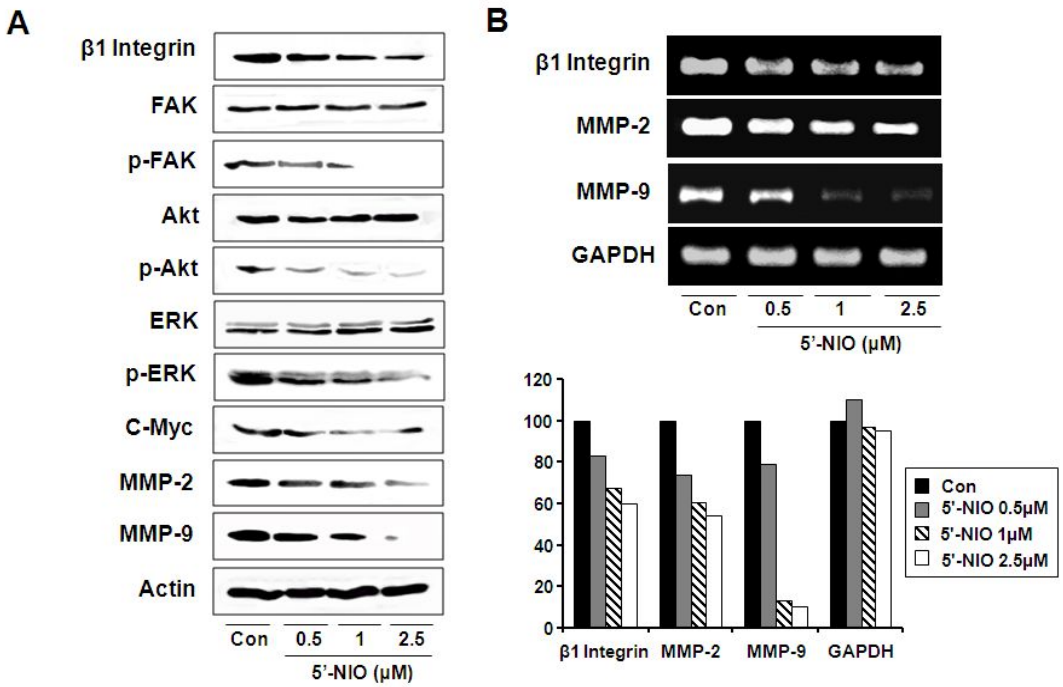


Fig. 4. Effect of 5′-NIO on β1 integrin/Akt signaling pathway. SGT cells exposure to different concentrations of 5′-NIO (0.5, 1 and 2.5 μM) for 24 h. (A) 5′-NIO was decreased β1 integrin, phosphorylation of FAK, Akt and Erk, down regulated the expression of MMP-2, MMP-9 in a dose-dependent manner. (B) Furthermore, mRNA measured by RT-PCR, and 5′-NIO suppressed MMP-2/-9 mRNA expression.

4. Effect of 5'-NIO on MMP-2/-9 secretion

Extracellular matrix breakdown is pivotal for cellular invasion, indicating that matrix-degrading proteinases are essential for tumor cell metastasis (38). Therefore, we determined the activity of MMP-2 and MMP-9 by gelatin zymography after 24 h incubation with 5'-NIO in SGT cells. As shown in Fig. 5, treatment of 5'-NIO suppressed the gelatinolytic activities of MMP-2 and MMP-9 of SGT cells in a dose dependent manner.

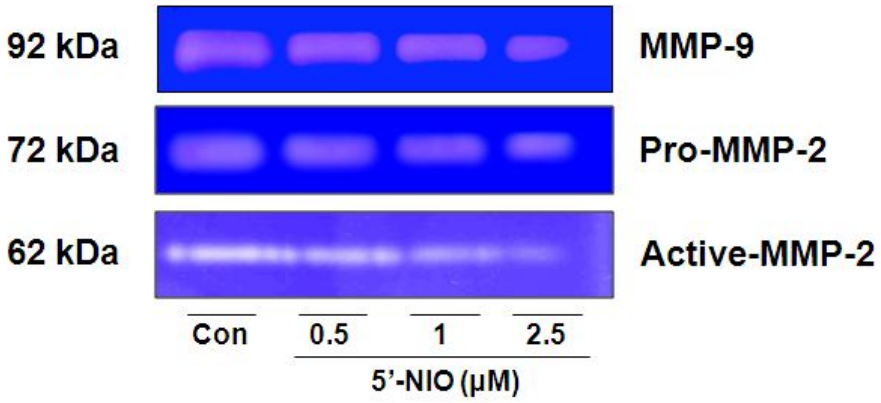


Fig. 5. Effect of 5′-NIO on MMP-2/-9 secretion. The cells were treated with 5′-NIO for 24 h. Each conditioned media was collected, and then was analyzed for the gelatinolytic activities by zymography. 5′-NIO decreased MMP-2/-9 secretion in a dose dependent manner.

5. Effect of 5'-NIO on SGT cell adhesion to type I collagen

The ECM is a complex mixture of matrix molecules which are typically large glycoproteins, including the fibronectins, collagens, laminins and proteoglycans (39). Among the ECM ligands, type I collagen is crucial for tumor cell adhesion and spreading (40, 41). To examine the influence of the ECM on SGT cells, we performed SGT cell attachment to type I collagen using adhesion assays (Fig. 6). SGT cell adhesion to type I collagen is significantly reduced by 5'-NIO in a dose dependent manner. These results suggest that adhesion to type I collagen of SGT cells in culture can be influenced through 5'-NIO.

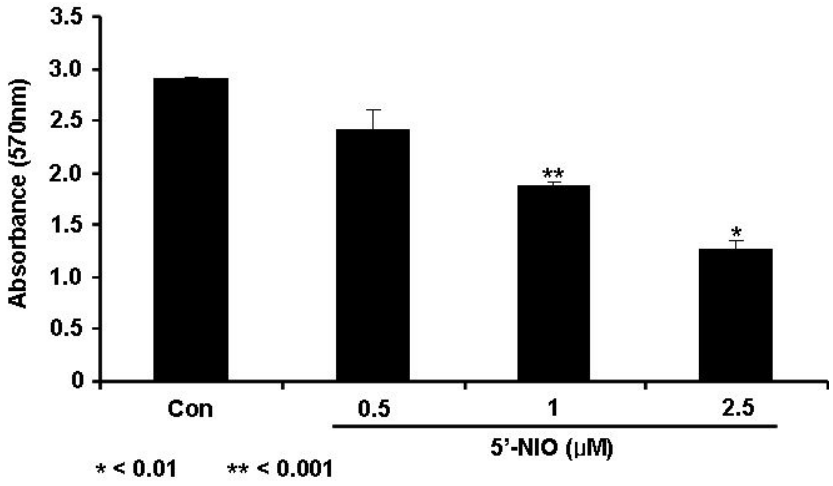


Fig. 6. Effect of 5′-NIO on SGT cell adhesion to type I collagen. Cell were treated with various concentrations (0.5, 1 and 2.5 μM) of 5′-NIO and were then subjected to analyses for cell-matrix adhesion as described in the MATERIALS AND METHODS. SGT cell adhesion to type I collagen was significantly reduced by 5′-NIO in a dose dependent manner.

6. Effect of 5'-NIO on protein expression after type I collagen treatment

To confirm the prior results, we measured effect of 5'-NIO on protein expression after type I collagen treatment. Treatment of type I collagen increased the expression of β1 integrin, MMP-2 and MMP-9, and phosphorylation of FAK compare with control (Fig. 7A). In addition, SGT cells with type I collagen treatment were exposed to different concentrations of 5'-NIO for 24 h. The expression of β1 integrin, MMP-2, and MMP-9 and phosphorylation of FAK was suppressed by 5'-NIO in a dose-dependent manner (Fig. 7B).

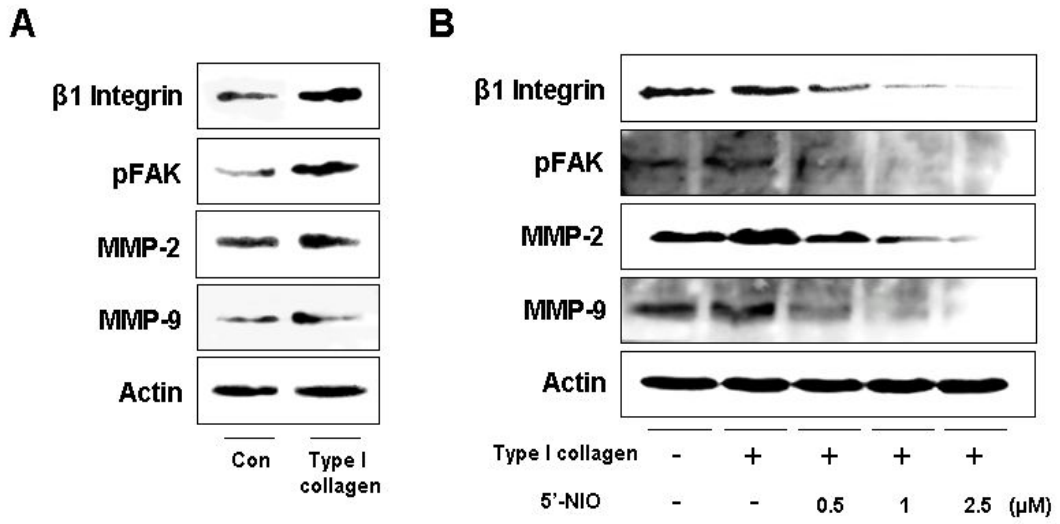


Fig. 7. Effect of 5′-NIO on protein expression after type I collagen treatment. (A) SGT cells exposure to type I collagen for 3 h. Type I collagen treated SGT cells was increased β1 integrin, phosphorylation of FAK, MMP-2, MMP-9 compare with control. (B) SGT cells exposure to different concentrations of 5′-NIO (0.5, 1 and 2.5 μM) for 24 h after type I collagen treatment for 3 h. 5′-NIO was decreased β1 integrin, phosphorylation of FAK, MMP-2, MMP-9 in a dose-dependent manner.

7. Effect of 5'-NIO on SGT cell migration after type I collagen treatment

Finally, we investigated whether 5'-NIO inhibits cell migration enhanced by type I collagen. SGT cells migration increased by type I collagen compare with control. And 5'-NIO suppressed the migration of SGT cells treated with type I collagen in dose dependent manner (Fig. 8).

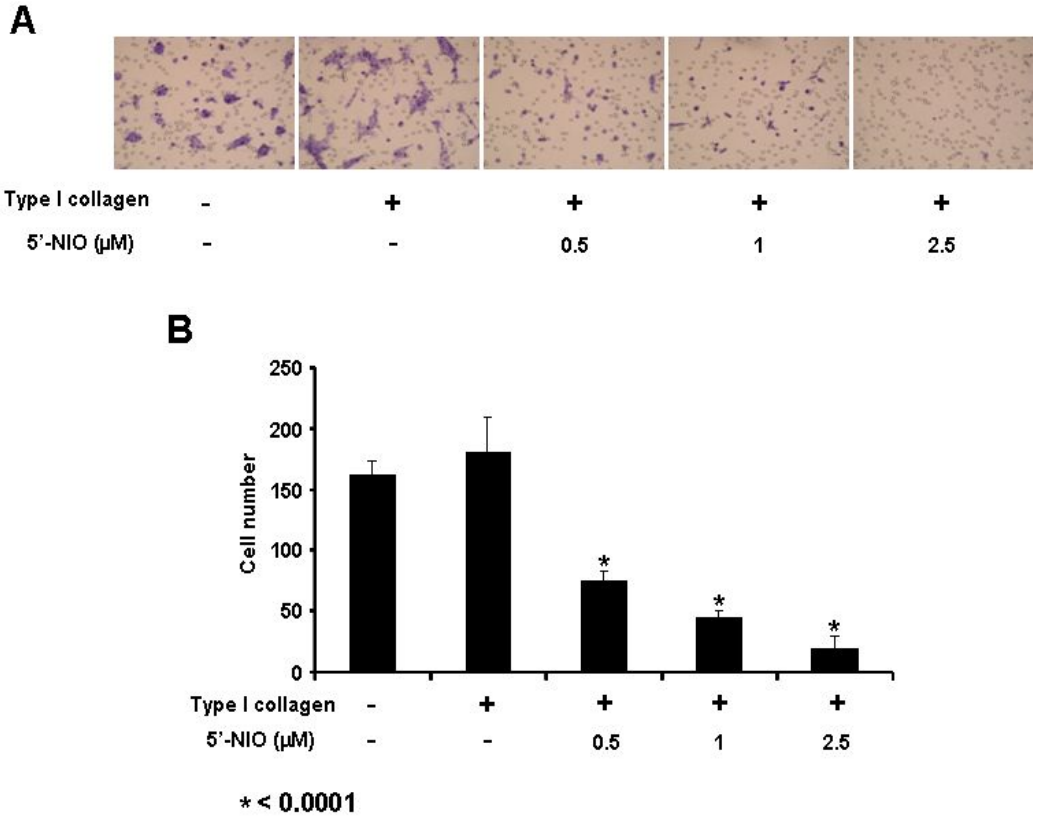


Fig. 8. Effect of 5′-NIO on SGT cell migration after type I collagen treatment. (A) The cells were cultured in the presence of various concentrations of 5′-NIO for 24 h after type I collagen treatment for 3 h within a migration chamber. Microscopy images detected cells that migrated into the inner membrane. (× 200) (B) The cell migration was quantified by counting the number of cells that migrated into the inner membrane. Compared with that of control, type I collagen increased SGT cells migration and 5′-NIO significantly decreased cell migration.

Ⅳ. Discussion

Cancer metastasis occurs by a multi-step process involving alterations in tumor cell adhesion, migration, and invasion (42). The major mechanisms of cancer invasion and migration are primary cancer cells disseminate and grow at a distant site resulting in a secondary tumor (43). Tumor invasion and metastatic spread are complex, multistep processes requiring a dynamic interaction of tumor cells with host environment. When cancer cells invade and migrate, various proteolytic enzymes contribute to the degradation of ECM (17, 18). Moreover, cells interact with the ECM proteins via transmembrane proteins, of which the integrin superfamily of cell surface receptors has been the most studied. Integrins are known to be crucial regulators of tumor cell interactions with the microenvironment. The β1 integrin subunit heterodimerizes with at least 12 different α subunits, creating receptors that mediate cell interactions with a wide variety of ECM ligands such as collagen, laminin, and fibronectin. These interactions, in turn, modulate diverse cell behaviors, including proliferation, survival, adhesion and motility. The β1 integrin has been studied extensively in the biology of solid tumors, and its expression has been shown to correlate with poor prognosis in cancers of the lung (44), pancreas (45), and cutaneous melanoma (46). The β1 integrin is also implicated in the progression and metastasis of human breast cancer (47, 48). Finally, signaling through the β1 integrin is thought to mediate resistance to chemotherapy and radiotherapy in lung, breast cancer, and hematologic malignancies (49-51).

5'-NIO is one of indirubin derivatives and reported that it has induced apoptosis and suppressed the cell growth through the arresting cell cycle in various types of human cancer cells (1, 4-8). Moreover, we have previously demonstrated 5'-NIO inhibits the tumor proliferation and induces apoptosis in a rat tumor model (3). However, the relationship between 5'-NIO and tumor migration and invasion has not yet to be established. In this study, we investigated whether 5'-NIO inhibits invasion and migration in salivary gland adenocarcinoma SGT cells.

First, we examined cell viability using the MTT assay and shown the cell viability of approximately 85% in 1 μM 5′-NIO (Fig 1). Thus, we treated cells with 0.5, 1 and 2.5 μM 5′-NIO in subsequent experiments. Next, we observed the effect of 5'-NIO in SGT cell invasion and migration using the invasion assay, wound-scratch and migration assay. We confirmed that 5'-NIO inhibits the invasion and migration compared with control (Fig 2 and 3). As previously reported, integrin-mediated signals can activate the Akt/Erk pathway and its subsequent signaling functions (24-28). It has been demonstrated that integrins induce phosphorylation of FAK, Akt and Erk. Moreover, these signals have been reported to directly involve the regulation of expression of MMP-2 and MMP-9 (52-54). Therefore, we investigated β1 integrin signaling pathway using western blot and RT-PCR. As expected, we showed β1 integrin is leading the phosphorylation of FAK, Akt, and Erk and down regulate the MMP-2 and MMP-9 (Fig. 4). Additionally, we showed 5'-NIO decreased MMP-2 and MMP-9 activity using gelatin zymography (Fig 5).

We also investigated SGT cell attachment to type I collagen using adhesion assays (Fig. 6). SGT cell adhesion to type I collagen is significantly suppressed by 5'-NIO in a dose dependent manner compare with control. To confirm the prior results, SGT cells exposure to type I collagen for 3 h and SGT cells treated with type I collagen increased expression of β1 integrin, phosphorylation of FAK, MMP-2 and MMP-9 compared with control (Fig. 7A). In addition, 5'-NIO decreased expression of β1 integrin, phosphorylation of FAK, MMP-2 and MMP-9 compare with type I collagen treatment in a dose dependant manner (Fig. 7B). To investigate whether 5'-NIO suppress to migrated cells by type I collagen, migration assays were performed in SGT cells treated with 5'-NIO. SGT cells migration increased by type I collagen compared with control. And 5'-NIO inhibited the migration of SGT cells treated with type I collagen in a dose dependant manner (Fig. 8). These results suggest that adhesion and migration to type I collagen of SGT cells in culture can be influenced through 5'-NIO.

In conclusion, invasion and migration was dependent on productions of MMP-2/-9 through β1 integrin, phosphorylation of FAK, Akt and Erk. As mentioned above,

under the influence of 5'-NIO decreased β1 integrin activation, which is decreased phosphorylation of FAK, Akt and Erk. Then these signaling molecules to blocked MMPs production and subsequent suppressed invasion and migration. Our data showed that 5'-NIO could inhibit the invasion and migration of human SGT cells via these mechanisms. Therefore, our results indicated that 5'-NIO may be a valuable anti-invasive drug candidate for salivary gland cancer therapy.

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저작물 이용 허락서

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논문제목	한글 : β1 integrin/Akt 신호전달을 타겟으로 하는 5'-Nitro-Indirubinoxime의 타액선암 세포 침습 억제 효과 영어 : 5'-Nitro-Indirubinoxime inhibits SGT cell invasion by targeting β1 integrin/Akt signaling pathway				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가
   없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(O) 반대( )

2009년 5 월 29 일

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