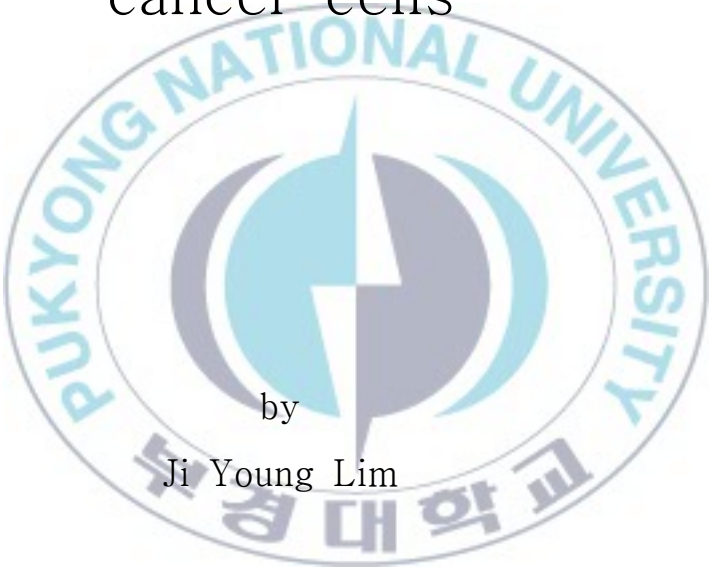
Thesis for the Degree of Master of Science

# Cytoskeletal reorganization and cell cycle arrest by 5-Aza-2'-deoxycytidine on 267B1/K-ras human prostate cancer cells




by

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August 2010

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(5-Aza-2'-deoxycytidine에 의한 267B1/K-ras cell 에서의 세포주기 억제와 세포골격의 조절)

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by

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# Cytoskeletal reorganization and cell cycle arrest by 5-Aza-2'-deoxycytidine on 267B1/K-ras human prostate cancer cells

A dissertation

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# ABSTRACT

The demethylating agent, 5-Aza-2'-deoxycytidine, has crucial value for the epigenetic therapies of cancers. Many studies have reported that 5-Aza-2'-deoxycytidine treatment inhibited Dnmts and induced cell proliferation and apoptosis in tumors for cancer therapies, but their exact *in vivo* mechanism is required more studies and results.

In this study, the results show that the 5-Aza-2'-deoxycytidine inhibits the cell proliferation in dose dependent manner. The cell proliferation is arrested at the inhibitory concentration of 10 μM and the exposure time was 72 h.

Also, 5-Aza-2'-deoxycytidine causes cell cycle arrest. Especially, Cyclin-dependent kinases (CDKs) and Cdc25A expression decreased remarkably in treated 267B1/K-ras.

Inhibition of DNA methylation, ultimately induce the recovery of tumor suppressor genes. Chimaerin in these genes has GTPase-activating protein (GAP) domain that targets Rac and is known to inhibiting expression of Rac. Through the experiment, it is confirmed that increased expression of Chimaerin by 5-Aza-2'-deoxycytidine down-regulates Rac-dependent cytoskeletal pathway on 267B1/K-ras cells.

These results represent that 5-Aza-2'-deoxycytidine causes the cell cycle arrest and regulates indirectly Rac-dependent actin polymerization of 267B1/K-ras cells. Therefore it would be appropriate candidate as potent anti-cancer drug.

# INTRODUCTION

Recently developed epigenomic technologies have made it possible to establish epigenetic differences between normal and cancer cells, thereby enhancing our understanding of the disease development (Fraga and Esteller, 2002).

Tumor specific methylation may provide means for detection and early diagnosis of cancer. If methylation of CpG islands are a critical parameter in tumor maintenance or progression, it would be desirable to reverse DNA hypermethylation (Pfeifer and Rauch, 2009; Yen *et al.*, 1992). Changes in DNA methylation patterns are one of the most frequent events that occur in human tumors and altered CpG methylation patterns discriminate tumor tissue from its nonmalignant counterpart tissue or normal adjacent tissue (Jones *et al.,* 2007). Therefore, demethylating agents are needed on epigenetic therapies for reestablish the expression of silenced tumor suppressor genes and clinical use against some cancers. The cytosine methylation is the most studied modification in epigenetics (Mulero-Navarro *et al.,* 2008). 5-Aza-2'-deoxycytidine is an inhibitor of DNA methylation, involved in cancer therapies but it has higher cytotoxic effects on normal cells.

5-Aza-2'-deoxycytidine of anti-drugs for epigenetic therapies potentially, an inhibitor of DNA methyltransferase, is associated with DNA and DNA methyltransferase. Deoxynucleoside analogues as 5-Aza-2'-deoxycytidine are converted into the triphosphate inside S-phase cell and are incorporated in place of base into DNA. Once in DNA, fraudulent bases from covalent bonds with DNA methyltransferases, result in the depleting of active enzymes

and demethylation of DNA (Egger *et al.*, 2004). 5-Aza-2'-deoxycytidine has been reported to induce cell cycle arrest and have anti-cancer activities, and shown to have significant cytotoxic and antineoplastic activities in many experimental tumors (Bender *et al.,* 1998; Natsume *et al.,* 2008).

Also, it induces to recovery of inhibited tumor suppressor genes in several human cancers. Gatekeepers such as oncogenes and tumor suppressor genes directly regulate cellular growth and differentiation pathways (Bishop, 1983). So, gatekeepers defects lead to abnormal cellular proliferation, differentiation and tumor.

Cathepsins have specific roles in physiological processes, including bone remodelling, epidermal homeostasis and antigen presentation (Turk *et al.*, 2002). They have also been implicated in many pathological processes including tumor invasion and metastasis (Sloane *et al.*, 1994; Kos and Lah, 1998).

DLC-1 was originally identified as a potential tumor suppressor. Loss of DLC-1 gene expression is found in a wide variety of human cancers including lung and breast (Durkin *et al.*, 2007). Recently, genome-wide sequencing analyses of human tumors have identified missense mutations in DLC-1 (Sjoblom *et al.,* 2006; Jones *et al.*, 2008).

Chimaerins are involved in many functions through Rac inactivation (Hall *et al.,* 2005; Yang and Kazanietz, 2003). With regard to β2-chimaerin, down-regulation of its message level was found in some types of cancers such as high-grade glioma (Yuan *et al.,* 1995) and mastocarcinoma (Menna *et al.,* 2003; Yang *et al.,* 2005), suggesting that reduced expression of this gene contributes to the development of the tumors.

Therefore, Reducing of Chimaerins in various types of cancers is important evidence about Chimaerins as a tumor suppressor (Bruinsma *et al.*, 2007). The interaction between Rac and Chimaerin in cancer signaling is intuitive, based on the role of Chimaerins to inhibit Rac that itself shows increased activity in a multiple of human carcinomas (Burbelo *et al.,* 2004; Fernandez-Zapico *et al.,* 2005; Jordan and Devi, 1999; Lozano *et al.*, 2003; Sahai and Marshall, 2002).

It is widely accepted that Rac and other Rho GTPase play critical roles in the regulation of cell morphology and movement, invasion, proliferation and malignant transformation which are all crucial events in cancer development and progression (Gomez *et al.*, 2005; Ridley, 2004). Cells move in response to signals and eventually affect cytoskeletal and adhesive structures of the cell. The point role of the cytoskeleton and its regulation makes the system weak for mutation and defects. Moreover, these may affect immune system or lead to tumor invasion and metastasis.

Typically, these processes, which characterize the cancer malignancy is associated with aberrant active cell migration (Lambrech *et al.,* 2004). Specially, Rac of GTPase families regulates lamellipodia formation through activation of the phospholipid metabolism that generates PI(4,5)-P, which in turn regulates several actin binding proteins such as WAVE, Arp2/3 and gelsolin. Lamellipodia is large veil-like sheets, which contain highly branched and cross-linked actin filament, also, it is generally associated with cell migration (Small, 1994).

This study shown the changes in 267B1/K-ras cells by treatments of 5-Aza-2'-deoxycytidine. Human epithelial cell line

(267B1) established from fetal prostate tissue can be malignantly transformed by a biological carcinoma, and can serve as a useful model for investigations of the progression steps of carcinogenesis. Activated K-ras was introduced into 267B1 cells by infection with the Kirsten murine sarcoma virus (Kim *et al.,* 2005). Thses cells are immortalized but contained the essential characteristics of primary human prostate epithelial cells such as morphology, expression of cytokeratins (Parda *et al.* 1993).

These changes, particularly, are confirmed in the arrest of cell cycle and regulation of actin cytoskeleton associated with Rac.

# MATERIALS AND METHODS

## Cell Culture

Transformed 267B1/K-ras was cultured in RPMI-1640 media (Hyclone) containing 10 % heat-inactivated fetal bovine serum (FBS) and penicillin-streptomycin (penicillin / 100 ㎍/㎖, streptomycin / 100 units/㎖ sterile filtered. Hyclone). And it was maintained at 37 ℃ in a 5 % $CO_2$ humidified atmosphere. For treatment with 5-Aza-2'-deoxycytidine (Sigma-Aldrich, USA), it was dissolved in dimethyl sulfoxide (DMSO, Fluka) and then kept at -20 ℃ Also it was enhanced every 24 hours per plate at final concentration 0 and 10 μM mixed new media for 72 h.

## Cell proliferation assay

$1 \times 10^4$ cells/㎖ were plated onto 96-well plates in duplicate, using 100 ㎕ per well, various concentrations of 5-Aza-2'-deoxycytidine (0, 10, 20, 50 and 100 μM) were treated after 72 h of incubation and 10 ㎕ of EZ-Cytox Cell Viability Assay Solution WST-1® (Daeil Lab Service, Jong-No, Korea) was added and incubated at 37 ℃ for 3 h. And then it was read at 460 ㎚ with ELISA (Molecular Devices, Silicon Vally, CA, USA).

### Expression Analysis

Expression levels of selected tumor suppressor genes were carried out through Real-time PCR. mRNA was purified using RNesay column (Qiagen, Valencia, CA.). The purified mRNA used for Reverse transcription PCR using MuLV reverse transcriptase. Quantitative assessments of DNA amplification were performed by fluorescence-based real-time detection system (TAKARA BIO INC., JAPAN) using SYBR Green.

### Cell cycle analysis

Cells were treated with 5-Aza-2'-deoxycytidine for 12 h and then harvested by trypsinization. Cells were washed with PBS and centrifuged 1800 rpm for 3 min, with cold ethanol overnight at 4 ℃. And RNase (10 ㎍/㎖) was treated and incubated at 37 ℃ for 1 h. Then the its cells were stained with 10 ㎕ of Propidium Iodide (Sigma-Aldrich, USA). The distribution of various stage of the cell cycle was analysed by flow cytometry (BD FACS Calibur CA, USA).

### Western blotting

The 267B1/K-ras cells were cultured and treated with 10 μM as mentioned above, and then harvested for western blotting. The harvested cells were collected by centrifugation, lysed with ice-cold lysis buffer ((50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1

mM DTT, 0.5 % NP-40, 1 % Triton X-100, 1 % Deoxycholate, 0.1 % SDS and proteinase inhibitors (PMSF, EDTA, Aprotinin, Leupeptin, Prostatin A) (Intron Biotechnology, Gyeonggi, Korea)). After incubation on ice for 30 min, the proteins were congregated by centrifugation at 14000 rpm for 20 min at 4 ℃. The protein contents was determined by a Protein Quantification Kit (CBB solution®) (Dojindo Molecular Technologies, Rockvile, MD, USA) with bovine serum albumin (BSA) as standard. An aliquot from each sample was boiled with sample buffer for 4 min and then electrophoresed on 12 % SDS-polyacrylamide gel-electrophoresis (SDS-PAGE). The proteins were electrotransferred to a nitrocellulose membrane (PALL Life Science, MI, USA). After transfer, the membrane was blocked in PBST buffer (135 mM Sodium chloride, 2.7 mM Potassium chloride, 4.3 mM Sodium phosphate, 1.4 mM Potassium dihydrogen phosphate, 0.5 % Tween-20) with 5 % skimmed milk powder for overnight at 4℃. The blots were probed with the primary antibodies (anti-Akt, anti-$p21^{waf1/cip1}$, anti-Cdc25A, anti-CDK2, anti-CDK4, anti-CDK6, anti-Rac1/2/3, anti-WAVE2, anti-Arp2/3 and anti-Villin1) (Cell Signaling Technology Inc., Beverly, MA, USA) (anti-CathepsinO and anti-Chimaerin2) (Abcam plc. Cambridge Science Park, Cambridge, UK) (anti-DLC1) (Snata Cruz Biotechnology Inc., CA, USA) and then washed 3 times in PBST, followd by incubation for 1 h with horseradish peroxidase-coupled anti-rabbit IgG or anti-mouse IgG as second antibodies (Cell Signaling Technology Inc.). The blots were then washed and the signals visualized by enhanced chemiluminescent (ECL) detection solutions (Pierce, Rockford, IL, USA).

### Immunofluorescence

Cells were cultured (37 ℃, 5 % $CO_2$) on coverglass-Bottom dishes (SPL LIFESCIENCES, Gyeonggi, Korea) in RPMI-1640 media (Hyclone, Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (FBS) and penicillin-streptomycin (penicillin / 100 ㎍/㎖, streptomycin / 100 units/㎖ sterile filtered. Hyclone, Logan, UT, USA) for 72 h. For this, cells were fixed with 4 % formaldehyde (JUNSEI Chemical Co., Ltd, JAPAN) 15 min at room temperature and blocked for 1 h in 5 % same normal serum of host of primary antibodies and 0.3 % Triton X-100. Fixed and blocked cells were incubated with 0.1 ㎍/㎖ of primary antibodies (anti-WAVE2 and anti-Villin1) (Cell Signaling Technology Inc., Beverly, MA, USA) for 3 h and then with 0.1 ㎍/㎖ of anti-rabbit IgG (H+L), F(ab') fragment (Alexz Fluor 488 conjugate) for 1 h. Stained cells were mounted of slides in Prolong Gold Antifade Reagent (Invitrogen, Eugene, OR, USA). Samples were examined under a Nikon ECLIPS 50i microscope equipped with charged-coupled device (CDD) camera. Images were captured and processed with Hign-Content Analysis software (Cambridge Healthtech Insti., Needham. MA. USA).

# RESULTS

## Inhibition of cell proliferation

The results of MTT assay showed that 5-Aza-2'-deoxcycytidine inhibit proliferation of 267B1/K-ras cells after 72 h of treatment. The observed inhibitory effect was in dose-dependent manner. The $IC_{50}$ value of 5-Aza-2'-deoxycytidine was 10 μM (Fig. 1).

Also, through the pictures of cells on several conditions, it is confirmed that the higher concentration and treated time, the more cells died and floated (Fig. 2). Thus, the $IC_{50}$ value, 10 μM of 5-Aza-2'-deoxycytidine was used in subsequent experiments.

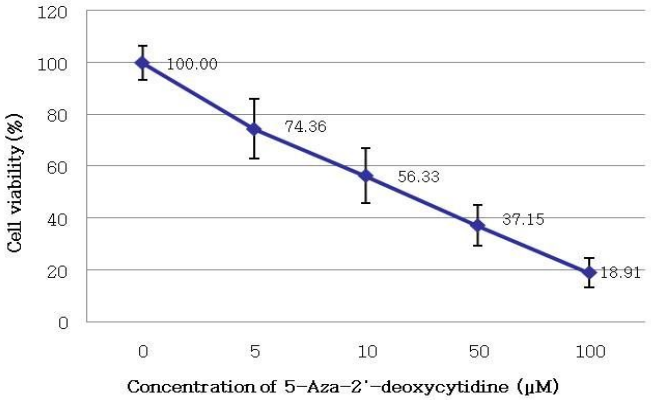
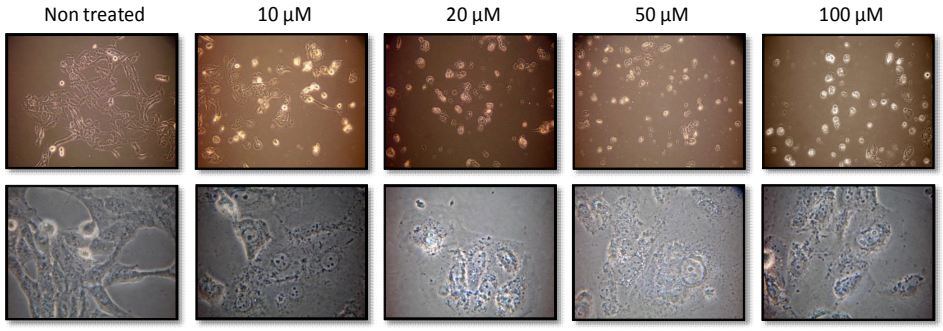



**Fig. 1. The effects of 5-Aza-2'-deoxycytidine on cell proliferation.**

Proliferation inhibition rates of 5-Aza-2'-deoxycytidine on 267B1/K-ras cells determined by MTT assay. The cells were treated with indicated concentrations of 5-Aza-2'-deoxycytidine for 72 h. 5-Aza-2'-deoxycytidine treatment inhibited the growth of 267B1/K-ras prostate cancer cells.

A)




B)

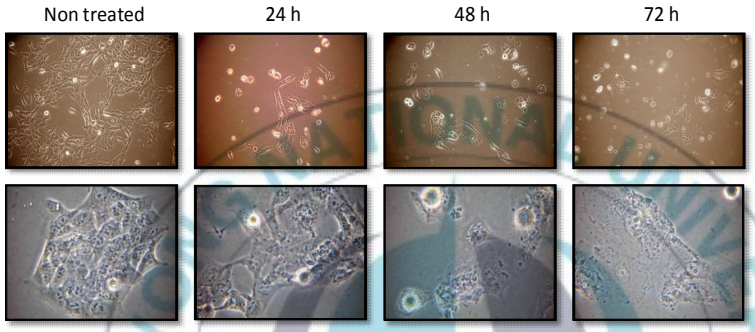



Fig. 2. Morphological changes in different treatment conditions of 5-Aza-2'-deoxycytidine.

Morphological changes of 267B1/K-ras cells depend on dose (A) and treatment time (B) of 5-Aza-2'-deoxycytidine. On panels A and B, the above pictures are magnified to 100 times, and others in the bottom are 400 times by a phase microscope.

## Cell cycle arrest

The results revealed that when the cells were treated with 10 μM of 5-Aza-2'-deoxycytidine for 72 h, it leads to changes in cell cycle progression and the expression of cell cycle regulator proteins such as Cyclin-dependent kinases (CDKs). Specifically, the expression of Cdc25A was completely arrested with increased expression of $p21^{Waf1/cip1}$ (Fig. 3). Tumor suppressor protein $p21^{Waf1/cip1}$ induces inhibition of cell cycle progression. It serves to inhibit kinases activity and block progression through G1 to S phase. The essential role of $p21^{Waf1/cip1}$ relies upon its well known ability to inhibit Cyclin-dependent kinases and DNA replication (Waga *et al.* 1994; Chuang *et al.* 1997) thereby inducing cell cycle arrest. Besides its classical roles, $p21^{Waf1}$ is also involved in a number of other specific functions that may also contribute to growth arrest.

Cdc25A regulates the G1 to S phase transition by dephosphorylation and activating the Cdk2/CyclinE and Cdk2/CyclinA complexes. The decreased expressions of CDK4, CDK6, CDK2 and Cdc25A were confirmed the arrest of cell cycle progression. Flow cytometry results showed that most of the cells accumulated in the G0/G1 phase and then the populations were decreased in the G2/M phase (Fig. 4).

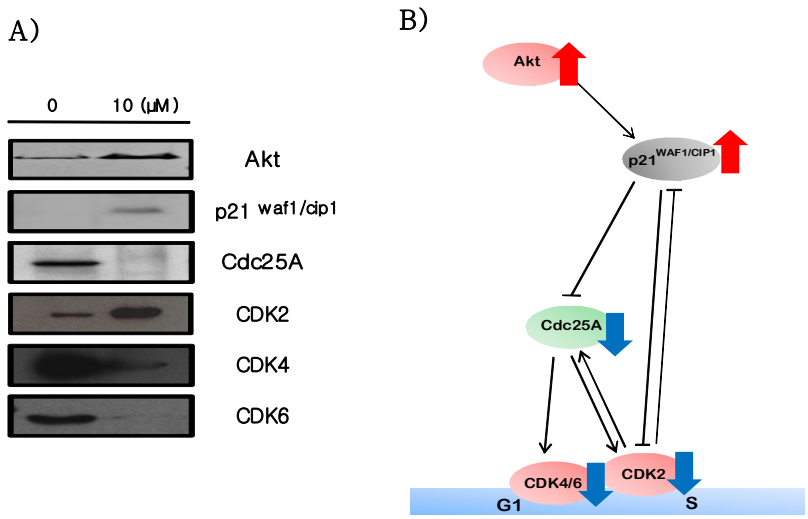



**Fig. 3. Expression levels of cell cycle regulatory proteins.**

The expression levels of proteins in cell cycle were examined by Western blot analysis (A). 5-Aza-2'-deoxycytidine treatment induced the arrest of G1-M phase in cell cycle (B.)

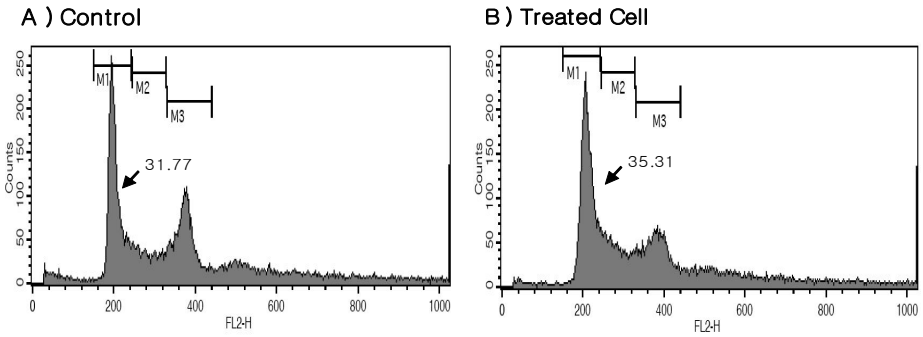



Fig. 4. FACS analysis for the cell cycle of 267B1/K-ras cells treated with 5-Aza-2'-deoxycytidine and stained by PI.

The degree of cell cycle arrest by 5-Aza-2'-deoxycytidine was analyzed by flow cytometry. G1(M1) areas of cells treated with 5-Aza-2'-deoxycytidine (B) increased compare to the untreated cells (A). Increasing of G1(M1) areas by treatment of 5-Aza-2'-deoxycytidine shows the arrest of G1 phase in Cell cycle. The result is from one representative experiment of three independent experiments that shown the similar patterns.

## Recovery of tumor suppressor genes

Inhibition of DNA methylation by 5-Aza-2'-deoxycytidine may cause the expression of tumor suppressor genes. To confirm this, Real-time PCR analysis was performed. The targeted genes are Cathepsin O (CTSO), Chimaerin2 (CHN2) and Deleted in Liver cancer 1 (DLC-1) for this analysis. Primer sequences and annealing temperatures are indicated in Table 1. The results showed that expression of CTSO and DLC-1 almost in same level and the remarkable change in CHN2 expression (Fig. 5). The same results were confirmed in protein level through immunoblotting (Fig. 6). From the above results confirm 5-Aza-2'-deoxycytidine cause the up-regulation of inhibited tumor suppressor genes in both gene and protein level.

Table 1. Primers and conditions used for real-time PCR

| Target gene | Sequences (5'→3') | Temperatures (℃) | Length of primer (bp) |
|---|---|---|---|
| Cathepsin O | CCACCGCCTTCTATGGAATAAAT | 60.2 | 23 |
| | CTTAACGGCAAAGACACATTGG | 60.0 | 22 |
| Chimaerin2 | TGTCCTCCGATGCTGAAGAAT | 60.9 | 21 |
| | CCGAGAGATGATCCCATGAAAC | 60.2 | 22 |
| DLC-1 | GGACACCATGATCCTAACAC | 48.4 | 20 |
| | CTCATCCTCGTCTGAATCGT | 51.2 | 20 |

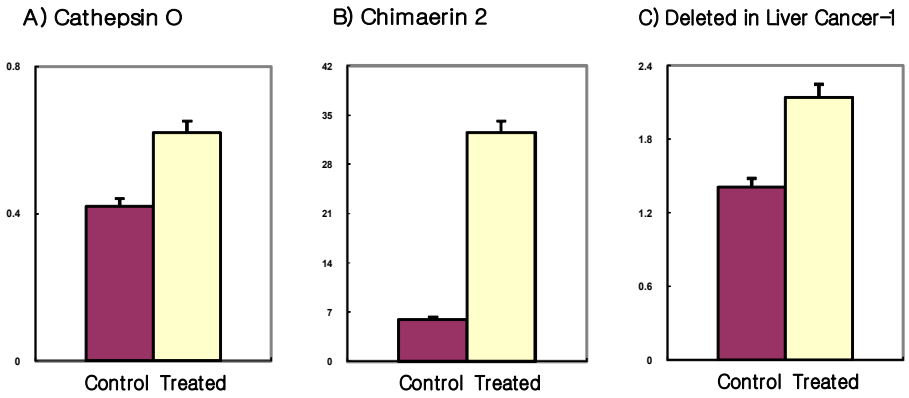



Fig. 5. Increased expression of tumor suppressor genes by 5-Aza-2'-deoxycytidine treatment in real-time PCR.

Targeted tumor suppressor genes were Cathepsin O (CTSO) (A), Chimaerin2 (CHN2) (B), and Deleted in Liver Cancer-1 (DLC-1) (C). When the cells treated with 10 μM of 5-Aza-2'-deoxycytidine, the expression levels of indicated genes were increased in real-time PCR experiments.

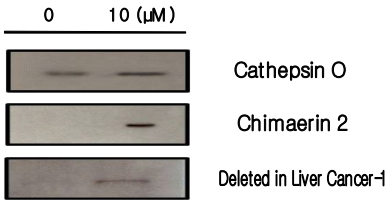



Fig. 6. Changes of targeted tumor suppressor gene in immunoblotting.

The same results were indicated in protein level through western blotting.

### Changes in Rac-dependent cytoskeleton

Chimaerin increased in both real-time PCR and western blot is widely accepted that it is inhibitor to Rac. In Fig. 6. The expression of Rac was decreased when 5-Aza-2'-deoxycytidine was treated at 10 μM for 72 h. With these results, member of the WASP/WAVE family of actin cytoskeletal regulatory protein, WAVE2 and Actin-Realted Protein, Arp and actin-binding protein, Villin are checked by western blot. As shown Fig. 7. the expressed WAVE2 and Villin1 were reduced, at the same time, the changes of cell morphology such as indistinct cell membrane and cell adjunction were observed by Immunofluorescence (Fig. 8.). This suggested that the recovery of Chimaerin by 5-Aza-2'-deoxycytidine treatments was controls the pseudopodia such as lamellipodia, also finally invasion of cancer cells can be regulated.

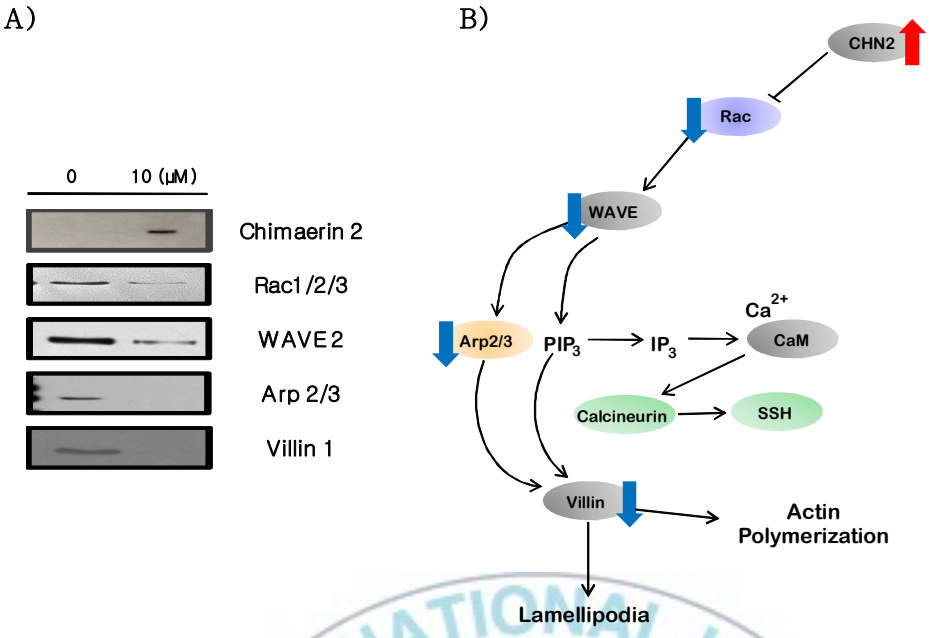



Fig. 7. Proposed signaling cascade of cytoskeleton modification mediated by Chimaerin2.

The expression levels of proteins in Rac-dependent cytoskeleton modification were examined by Western blot analysis (A). Increased expression of Chimaerin2 by 5-Aza-2'-deoxycytidine regulates Rac-dependent cytoskeleton modification on 267B1/K-ras human prostate cancer cells (B).

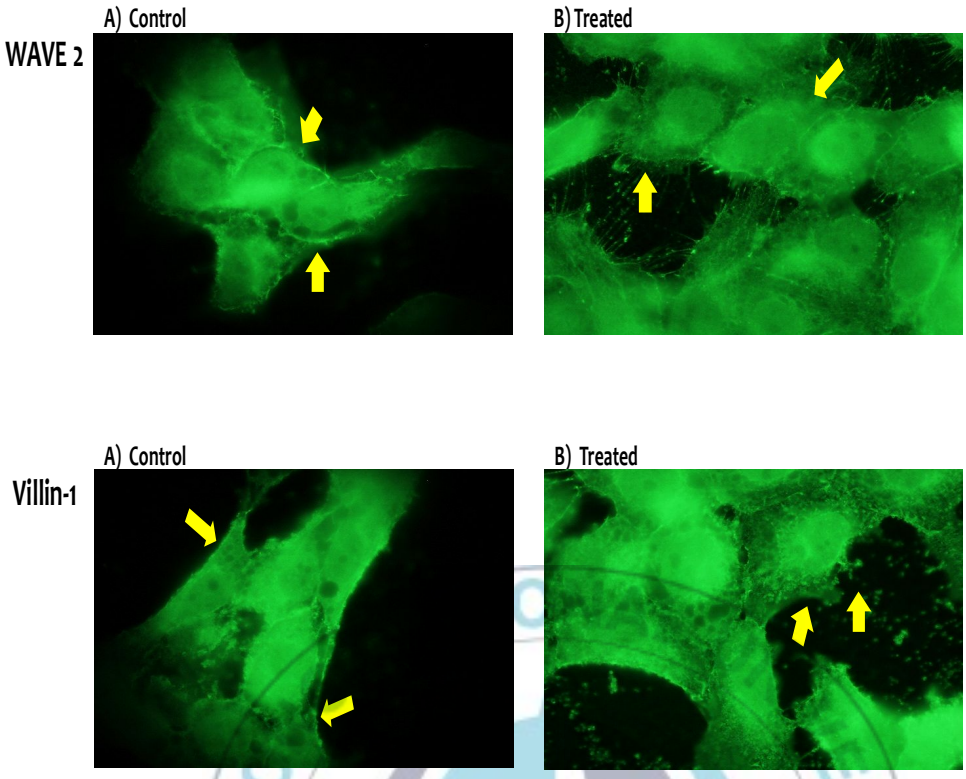



Fig. 8. Cell morphology by changes of WAVE and Villin-1 using immunofluorescence.

Target genes in Rac-dependent cytoskeletal modification pathway, WAVE and Villin effected to changes in cell morphology. As shown treated cells (B), cell shape is indistinct and adjunction of cell to cell was come off.

# DISCUSSION AND CONCLUSION

Because epigenetic therapies have raised considerable interests in cancer, 5-Aza-2'-deoxycytidine was targeted and studied. Many studies have reported that 5-Aza-2'-deoxycytidine treatment inhibited Dnmts and induced cell proliferation and apoptosis in tumors for cancer therapies, but their exact *in vivo* mechanism was required more studies and results.

In this study, the results showed that the 5-Aza-2'-deoxycytidine inhibits the cell proliferation in dose dependent manner. The cell proliferation was arrested at the inhibitory concentration of 10 μM and the exposure time was 72 h.

The progression of cell proliferation encounters the cell cycle check points. Cyclin-dependent kinases (CDKs) are an internal part of the cell cycle control machinery that regulator the proliferation of eukaryotic cells. Inhibition of Cdc25A by 5-Aza-2'-deoxycytidine arrests the G1-S phase transition. CDK2, CDK4 and CDK6 are important regulator in the control of how cells leave the quiescent state and transgress through the G0/G1 restriction point. The essential role of $p21^{Waf1}$ relies upon its well known ability to inhibit CDK and DNA replication, thereby inducing cell cycle arrest. Plus, $p21^{Waf1}$ is recruited to the Cdc25A to inhibit its activation (Vigneron *et al.*, 2006).

Cdc25A is a tyrosine phosphatase that functions as a key regulator in cell cycle progression and DNA damage response in eukaryotes. Cdc25A regulates the G1 to S phase transition by

dephosphorylating and activating the Cdk/CyclinE and Cdk2/CyclinA complexs (Zhang, 1999). Also, Cdc25A was reported that the expression is up-regulated in human prostate cancer (Chiu *et al.*, 2009). Flow cytometry result accumulation of cells in G1 phase and decreased population in G2 phase.

Inhibition of DNA methylation, ultimately induce the recovery of tumor suppressor genes. Real-time PCR analysis showed the higher expression of Cathepsin O (CTSO), Chimaerin2 (CHN2) and Deleted in Liver Cancer-1 (DLC-1). Expression of Chimaerin2 is remarkably increased the regulation of cytoskeleton dependent on Rac pathway. It acts as GTPase-activating protein (GAP) for Rac and inactivates Rac signaling, cell proliferation and cytoskeletal reorganization by promoting the GDP-bound form and as tumor suppressor gene. Many studies have proposed a role for Rac in the control of mitogenesis through its ability to regulate G1-S transition and CyclinD1 expression. Moreover, Rac and other members of the Rho GTPase family are overexpressed in human tumors and hypermethylation of Rac leading to a higher rate of cell proliferation has been found in cellular models of human cancer (Yang *et al.*, 2005).

Similarly, downstream molecules such as WAVE of member in Wiscott-Aldrich Syndrome proteins, Actin-related protein (Arp) and Villin in Rac-dependent pathway were experimented. WAVE mediates actin dynamics by activating the Arp2/3 actin nucleation complex in response to activated Rho family GTPase. And Villin of member in gelsolin family has function about regulation of cell shape in response to external stimulation. Increased expression of these genes were seen in breast cancers, colorectal cancers

(Wang *et al.,* 2008; Sossey-Alaoui *et al.*, 2002).

Cytoskeletal changes are the key mechanism for metastasis in cancer cells. Regulation of Rac-dependent pathway by 5-Aza-2'-deoxycytidine represents to potential possibility besides inhibition of cell proliferation and induction of apoptosis.

In conclusion, the result of the present study showed that the anti-cancer effect of 5-Aza-2'-deoxycytidine was stronger. The most likely mechanisms of actin are the arrest of the cell cycle, regulation of cytoskeleton and inhibition of DNA methylation. This study may offer possible molecular bases for further research in the prostate cancer.

# 5-Aza-2'-deoxycytidine에 의한 267B1/K-ras cell에서의 세포 주기 억제와 세포 골격의 조절

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## 요 약

최근 암세포와 정상세포 사이의 epigenetic difference를 이용한 epigenomic technology가 cancer therapy로 발전하고 있다. 특히, DNA methylation은 tumor suppressor gene과 같은 유전자들의 발현을 억제하여 cancer development의 중요한 mechanism이 되고 있다. 이에 따라, DNA methylation의 억제제인 5-Aza-2'-deoxycytidine이 새로운 치료제로 연구되고 있다. 본 연구에서는 이러한 5-Aza-2'-deoxycytidine을 이용하여 전립선 암세포인 267B1/K-ras에서 어떠한 항암적 효과를 나타내는지 확인하였다.

그 결과, 5-Aza-2'-deoxycytidine은 10 μM의 농도에서 267B1/K-ras의 성장 억제를 유도함을 확인하였다. 또한, Cyclin-dependent kinases (CDKs), Cdc25A, $p21^{waf1/cip1}$과 같은 세포주기 조절자들의 발현을 변화시켜 Cell cycle arrest를 가능하게 하였다.

또한, 5-Aza-2'-deoxycytidine은 발현이 억제되어 있던 tumor suppressor gene들을 회복시킨다. 특히 이들 중 Chimaerin2는 tumor suppressor gene일 뿐만 아니라, 세포의 형태와 actin dynamics, 세포의 이동, 전이 등을 조절하는 Rac의 inhibitor로도 알려져 있다. 즉, 5-Aza-2'-deoxycytidine에 의한 Chimaerin2의 증가는 Rac의 발현을 저해하여 전립선 암세포의 이동과 전이를 간접적으로 조절할 수 있었다.

이러한 결과들로 부터, 5-Aza-2'-deoxycytidine이 전립선 암세포인 267B1/K-ras에서 cell cycle의 억제를 유도함은 물론이고, tumor suppressor gene의 회복과 함께 세포 골격의 변화를 통한 암의 전이 억제제로서의 가능성을 가지고 있음을 알 수 있었다.

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4년이라는 긴 시간을 정말 소중하고 행복하게 보낼 수 있게 해 주었던 우리 세포신호전달연구실의 인간승리 진수선배, 너무 착해서 큰일인 성자언니, 실험실을 언제나 즐겁게 만들어 준 현일선배, 덜 진지하면 더 좋을 상보선배, 몇 안남은 절친 동기 민재, 내 오른팔 미정이, 언제나 해맑은 순진이, 웃는게 멋진 석천이, 이제 막 실험실 생활 시작하는 해진이, 마냥 예쁘고 귀여운 막내들 초원이와 난희, 그리고 우리 세포방을 거쳐 간 여러 선배들과 후배들 모두 정말 저에게 큰 힘이 되어줘서 너무나 고맙고 사랑합니다.

지금은 각자 다른 곳에서 열심히 생활하고 있는 든든한 나의 친구들 윤정이, 고은이, 유진이, 보람이, 뒤늦게 학구열에 불타고 있는 용호, 가끔 타지 생활에 지칠 때면 언제나 따뜻하게 반겨주던 대구 친구들 은경이, 현진이, 진숙이, 효영이, 영원한 후배 경명이, 창은이 까지 너무나 고맙습니다.

마지막으로 어떤 일이 있더라도 믿어주시고, 사랑으로 응원해 주신 나의 가장 소중한 아빠, 엄마, 투덜대면서도 언제나 누나 말은 다 들어주는 착한 내 동생, 정제에게도 저의 마음을 다 전할 수는 없겠지만 진심으로 감사하며 이 조그만 결실을 바칩니다.

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