Effect of a new drug Ra-12a and Non-invasive quantification of tumor vessel with dynamic MR imaging in murine model.

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Directed by Professor Seung Hoon Choi

The Master's Thesis
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Ki Chong Park
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This certifies that the Master's Thesis of Ki Chong Park is approved.

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Sincerely,

K.C. Park.

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Effect of a new drug Ra-12a and Non-invasive quantification of tumor vessel with dynamic MR imaging in murine model.

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Cancer needs some blood vessel and MMPs(matrix metalloproteinases) for proliferation. Angiogenesis is an important step in the development of cancer that is necessary for primary tumor growth, invasiveness and metastasis.

Vascular endothelial growth factor (VEGF) is believed to be important for the process of initiation of angiogenesis and is a major mediator of cancer angiogenesis. Overexpression of VEGF has been shown in frequently various cancers.

Ra-12a can reduce to VEGF that represses cancer activity. vascular endothelial growth factor(VEGF), belonging to theplatelet-derived growth factor PDGF/VEGF family of growth factors, is a key regulator of angiogenesis.

VEGF is synthesized by numerous cell lines and secreted through conventional pathways. To date, six human VEGF mRNA species, encoding VEGF isoforms of 121, 145, 165, 183, 189 and 206 amino acids, are produced by alternative splicing of the VEGF mRNA. An important biological property that distinguishes the different VEGF isoforms is their heparin and heparan-sulphate-binding ability.

Ra-12a have ability to activation tissue inhibitors of metalloproteinases (TIMPs). to be activated TIMPs regulate matrix metalloproteinase (MMP) activity controlling the breakdown of extracellular matrix components and, thus, play an important role in the process of invasion and metastasis.

Moreover, here are several new functions, growth control, apoptosis, and anti-angiogenesis, in which TIMPs seem to be involved. The aim of this study was to elucidate the role of

TIMP-2 in human cancer assessing TIMP-2 protein expression in breast cancer evaluating its importance relative to manipulation cancer proliferation..I performed non-invasive d-MRI method in murine model. then, I will compared with angiogenesis and TIMP2 activity of control and experiment group.

The mouse was placed in a prone position and microscopy coil was placed at tumor area. T2-weighted image (T2WI, TR/TE=3367/80, matrix=256 x 256, 3mm slice thickness, 4 NEX) was acquired for detection of the tumor.

After the selection of single slice in the center of the map imaging, dynamic T1 gradient dual echo sequence (1000 phases, TR/TE/ = 10/2ms/30, temporal resolution = 1.28 sec, FOV = 20cm, slice thickness=5mm, matrix size=128128) was started 10-15 sec prior to contrast injection and acquired.

Key words :MMPs(matrix metalloproteinases),
TIMP2(Tissue inhibitors of metalloproteinase 2),
VEGF(vascular endothelial growth factor), Ra-12a
d-MRI(dynamic-MR Imaging)

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I. INTRODUCTION

New drug Ra-12a can inhibition to cancer it is histone deacetylase (HDAC) inhibitor.

Histone deacetylase (HDAC) inhibitors have emerged recently as promising anticancer agents. They arrest cells in the cell cycle and induce differentiation and cell death. The anti-tumor activity of HDAC inhibitors has been linked to their ability to induce gene expression through acetylation of histone and nonhistone proteins.

Local remodeling of chromatin is a key step in the regulation of gene expression, consequently affecting many cell functions (1).

One important mechanism in chromatin remodeling is the post-translational modification of the NH2-terminal tails of histones by acetylation (1, 2).

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are recruited to their target promoters through a physical interaction with a sequence-specific transcription factor (TF). They usually function within a multimolecular complex (enzymatic complex), in which the other subunits are necessary for them to modify nucleosomes around the binding site. These enzymes can also modify factors other than histones (protein X) to regulate transcription. Note that the position of the modified nucleosome that is shown has been chosen at random for this figure (Fig 1).

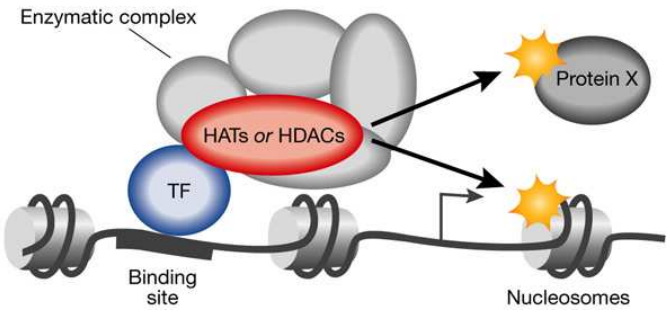
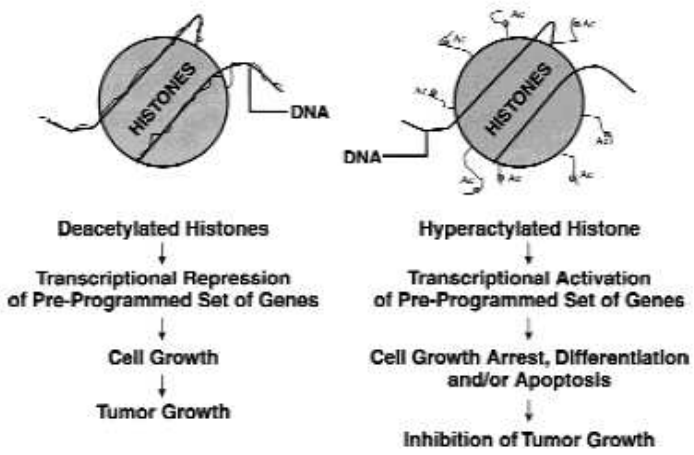


Fig 1. Model of local action of histone HATs (acetyltransferases) and HDACs (histone deacetylases).

Because of the importance of histone acetylation in chromatin function, HATs and HDACs have major roles in the control of cell fate and their misregulation is involved in the development of some human tumours. Consistent with the importance of HATs and HDACs, they are tightly regulated in living cells and their activity is modulated by signalling pathways (1).

Many reviews have focused on the various HAT and HDAC families and their roles in chromatin function, transcriptional regulation or cell fate, none has extensively explored how their function can be regulated (Fig 2 A, B).

(A)



(B)

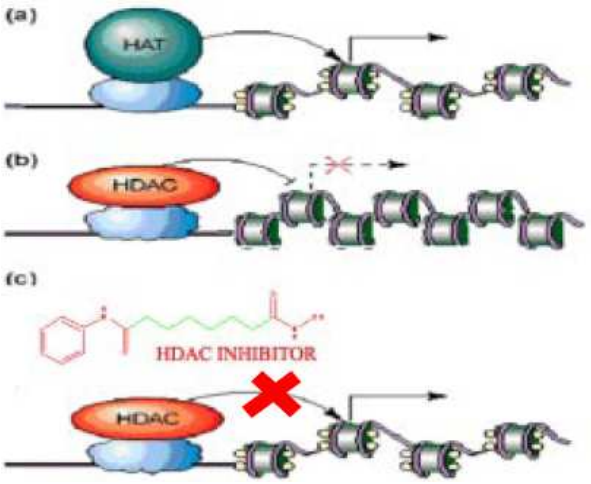


Fig 2. HDAC inhibitors induce tumor growth arrest, differentiation, apoptotic cell death.

Proposed mechanism of action of histone deacetylase (HDAC) inhibitors that induce tumor growth arrest, differentiation, apoptotic cell death. With inhibition of HDAC, histones are acetylated (Ac), and the DNA that is tightly wrapped around a deacetylated histone core relaxes. I propose that the accumulation of acetylated histones in nucleosomes leads to expression of specific genes, which, in turn, lead to cell growth arrest, differentiation, apoptotic cell death and, as a consequence, inhibition of tumor growth (Fig 2 A, B).

In transcriptionally silent chromatin, the histones that comprise the nucleosomes have low levels of acetylation on the lysine residues of their NH2-terminal tails (1, 2).

However, acetylation of these histone proteins neutralizes the positive charge on the lysine residues thereby disrupting nucleosome structure. This allows unfolding of the associated DNA and subsequent access by transcription factors leading to changes in gene expression (1, 2).

Acetylation of core nucleosomal histones is regulated by histone acetyltransferases and histone deacetylases (3, 4). A controlled balance between histone acetylation and deacetylation seems essential for normal cell growth and aberrant HDAC activity has been associated with the development of certain human cancers (4).

Perturbations in histone acetylation have been associated with a number of well characterized oncogenes and tumor suppressor

genes prompting the development of histone deacetylase inhibitors (HDAC-I) as a strategy for treating cancer (3, 4).

This emerging class of anticancer drugs can induce growth arrest, differentiation, and apoptotic cell death in many different types of tumor cells in vitro and in vivo (4, 5).

To invade and metastasize, cancer cells must penetrate the ECM (extracellular matrix) barriers in a process involving, among other factors, the proteolytic degradation of ECM components (6). MMPs (matrix metalloproteinases) consist a family of at least 16 structurally related enzymes capable of degrading ECM components (7). Once activated (8), the MMPs are subject to control by endogenous inhibitors such as 2 macroglobulin and more specifically by TIMPs (tissue inhibitors of metalloproteinases) (9).

Four different TIMPs have been described: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 (9). TIMPs are low molecular weight secreted proteins that bind to the active form of the MMPs at a 1:1 stoichiometric ratio, inhibiting enzymatic activity (9, 10).

TIMP-2 is composed 21,000 protein that forms a noncovalent stoichiometric complex with both the latent and active forms of MMP-2 (11).

Although in general TIMPs inhibit MMPs, TIMP-2 binds selectively to pro-MMP-2 (11, 12).

It has been reported that although the NH2-terminal domain of

TIMP-2 binds to the NH2-terminal domain of active MMP-2, the COOH-terminal domain of TIMP-2 binds specifically to the COOH-terminal domain of pro-MMP-2 (Fig 3) (13).

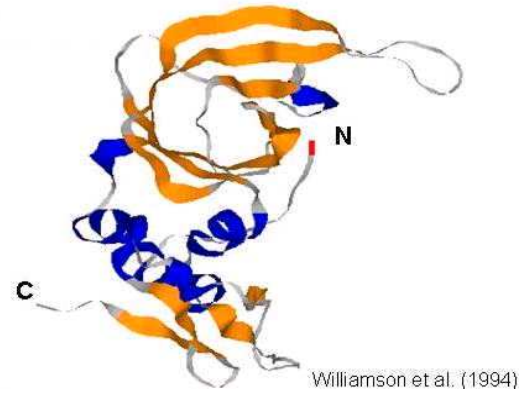


Fig 3. 3D-structure of TIMP2

This pro-MMP-2/TIMP-2 complex can still be activated and shows proteolytic activity (14).

There is also evidence that MT-MMP acts as a receptor for TIMP-2 and can complex with pro-MMP-2 leading to subsequent activation of pro-MMP-2 (15).

According to previous studies, MMP-2 protein seems to play an important role in urothelial cancer progression, because its levels have been associated with high-grade and high-stage carcinomas, as well as with recurrence of patients with advanced urothelial carcinomas after complete resection (16, 17).

These interactions still remain a controversial matter, and this

controversy is reflected in the results of studies investigating the role of TIMP-2 expression in the progression of various carcinomas.

At first, TIMP-2 was considered as a suppressor of invasion and metastasis because of the general concept that TIMPs inhibit MMPs (18-21).

However, the complexity of TIMP-2/MMP interactions in combination with the multiple new functions that have been attributed to TIMPs, for instance, cell growth control, relation to apoptosis, and angiogenesis (22-25), have led to a reconsideration of the role of TIMP-2 in cancer (26). In fact, there are several recent studies demonstrating a relation between TIMP-2 expression and progression of carcinomas (26-30).

Angiogenesis is an important step in the development of cancer and is necessary for primary tumor growth, invasiveness, and metastasis (31).

As the tumor grows, oxygen and nutrient demand increases.

Tumor cells release cellular signals to initiate the growth of new blood vessels to supply them with oxygen (Fig 4).

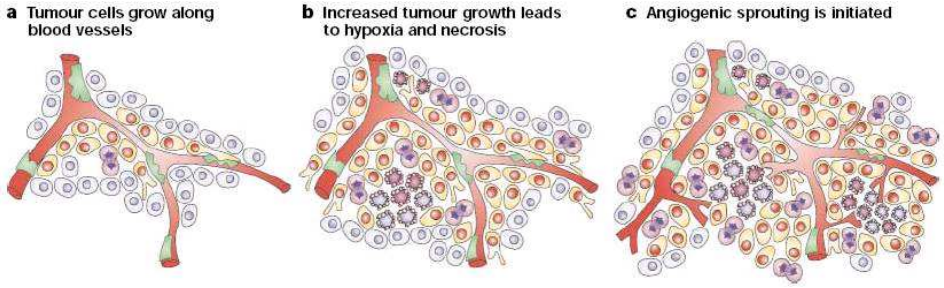


Fig 4. Angiogenesis and Cancer

Vascular endothelial growth factor (VEGF) is believed to be important for the process of initiation of angiogenesis and is a major mediator of breast cancer angiogenesis (32). Overexpression of VEGF has been shown in various cancers (33).

II. MATERIALS AND METHODS

1. Cell culture for Animal cancer model

A. Cell culture

FM3A cells are mammary caricinoma, female mouse and oubling proliferation time is 10~12 hour.

These cells were cultured in RPMI 1640 medium supplemented with 10% calf serum, 2 ㎎/㎖ sodium bicarbonate, 10% penicillin streptomycin and 5% CO_2 incubator at 37℃.

B. Animal model

C3H/HeJ-FasL (6week) mice are used as a general purpose strain in a wide variety of research areas including cancer, immunology and inflammation, sensorineural, and cardiovascular biology research. FM3A breast cancer cells (1.0×10^7) were injected in mouse sub-membrane (Fig 5).

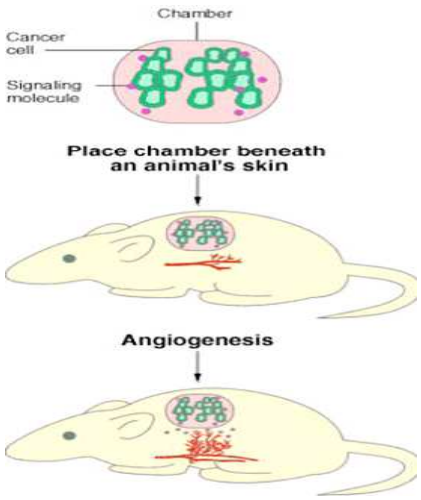


Fig 5. Angiogenesis in mouse

Drug injection performed (1 time per 2 days total 6~7 times) when complete cancer size (0.7cm x 0.7cm). Three kinds of drug LD50 is BA (170 ㎍/㎖), Ra-12A (206 ㎍/㎖), SAHA (138 ㎍/㎖) (Fig 6).

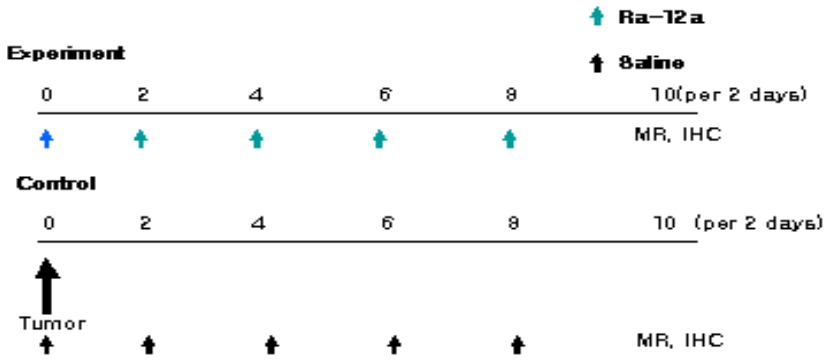


Fig 6. Experimental model-1.

Cultured in-vitro cancer cell line ($1*10^7$/1 mouse) is injected into mouse sub-membrane. When cancer cell is became complete tissue, beginning drug injection of LD50 (Fig 7).

Fig 7. Experimental model-2.

2. analysis of total protein

A. SDS PAGE & Western blot analysis

Frozen breast cancer samples were thawed at room temperature (RT), minced, weighed, placed in PBS (1 : 4w/v), and homogenized with a Tissue-Tearor (Biospec, USA).

The homogenate was sonicated three times for 20 sec at RT and centrifuged at 12,000 rpm for 10 min. The supernatant was diluted with electrophoretic sample buffer to obtain a protein concentration of total protein 39.2 $\mu g/4\mu\ell$ (9.8 $\mu g/\mu\ell$) then heated at 100C for 7 min. The heated samples were electrophoresed under denaturing conditions in sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) using a discontinuous procedure (34). Stacking gels were 4.5% polyacrylamide and separating gels were 7.5% polyacrylamide. Paired mini-gels (Mini-protein II cell, Bio-Rad Laboratories, U.S.A.) were loaded with 39.2μg protein per well. The protein concentration was estimated using the method of Bradford(35). Samples containing standard markers of TIMP2 (83kb) were run at 100 Volts/gel slab. After electrophoresis, one mini-gel was routinely stained by the Coomassie bluestaining method and the other was equilibrated in a transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol (v/v), pH 7.3). The proteins were then electrotransferred in the transfer buffer to a PVDF transfer

membrane (immuno-blotTM PVDF membrane for protein blot analysis 0.2μm , Bio-Rad) 1 hour at RT and 50 Volts.
To visualize the transferred proteins, the PVDF membrane was stained with Brilliant Blue R-250 (Sigma, St. Louis, MO) for 10 min and subsequently incubated in TBS (50 mM Tris/HCl, 20 mM NaCl, pH 7.4) containing 5% BSA(bovine serum albumin) or TBS-T (TBS with 0.1% Tween 20) with 5% skim milk for 1 hours at RT to block non-specific sites. The blot was then rinsed with TBS-T (TBS with 0.1% Tween 20).
The TIMP2(Ab-1) and β-actin(Ab-1) bindings were detected by incubating the membrane in a moist chamber overnight at 4C, with the primary antibody mouse anti-TIMP2(1:100 in dilution, Biomeda) and β-actin(1:400 in dilution, Biomeda).
After washing in TBS-T, the membrane was incubated with the second antibody (1 : 20000, anti-mouse IgG horseradish peroxidase conjugate dilution, Biomeda) for 2 hrs at RT.
The peroxidase reaction was developed with Amersham ECL reagents (Amersham Biosciences, USA). After imaging, the membranes were stripped and reprobed using monoclonal anti-beta-actin antibody as the primary antibody (Sigma, USA).

B. ELISA (Enzyme-Linked Immno-Sorbent Assays)

Vascular endothelial growth factor (VEGF) analysis by ELISA. we prepared total protein about cancer.

Total protein is extracted and tested for VEGF by Quantakine mouse VEGF ELISA according to the manufacturers instructions (R & D Systems, MN). Results were expressed as $\mu g/\mu \ell$ of extracted total protein.

Firstly, mouse VEGF microplates is two 96 well polystyrene microplates (12 strips of 8 wells) coated with polyclonal antibody specific for mouse VEGF and add total protein.

Secondly, mouse VEGF conjugate used a polyclonal antibody against mouse VEGF conjugated to horseradish peroxidase with preservatives.

Mouse VEGF Standard is 2.5 ng of recombinant mouse VEGF in a buffered protein base with preservatives, lyophilized.

Mouse VEGF Control of recombinant mouse VEGF in a buffered protein base with preservatives, lyophilized.

The concentration range of mouse VEGF after reconstitution is shown. The assay value of the control should be within the range specified on the control label.

Thirdly, wash buffer concentrate a 25-fold concentrated solution of a buffered surfactant with preservative and add stabilized hydrogen peroxide, stabilized chromogen

(tetramethylbenzidine).

Finally, adding stop solution a diluted hydrochloric acid solution. and determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

If wavelength correction is available, set to 540 nm or 570 nm.

If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm.

This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

C. Dynamic MRI

MR imaging were acquired by using a 1.5T MR scanner (Gyroscan Intera; Philips Medical Systems, Best, Netherlands).

In each tumor the perfusion and vascular permeability were measured by using bolus intravenous injection of 0.2 mmol/kg Gd-DTPA (Magnevist, Schering, Germany) mixed with 0.3ml of saline.

Mouse were anesthetized by intramuscular injection of ketamine (20 mg/kg) mixed with Rompun (20 mg/kg), and then a 26 gauge needle was inserted and maintained into the tail vein for injection of contrast agents (Fig 8-1).

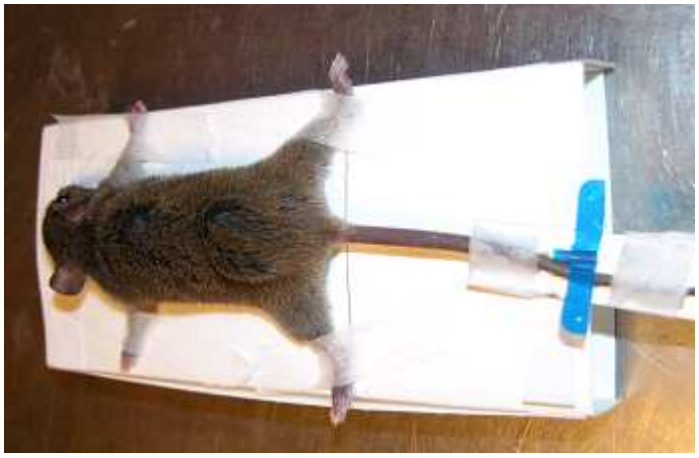


Fig 8-1. Prepare for mouse before to scan.

The animal was placed in a prone position and microscopy coil was placed at tumor area (**Fig 8-2, 8-3).**

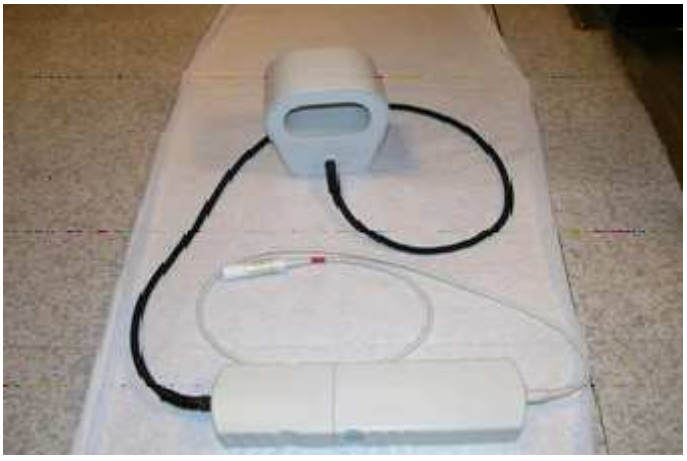


Fig 8-2. It's called 'coil' for small animal D-MRI.

Fig 8-3. over the setting for D-MRI.

T2-weighted image (T2WI, TR/TE=3367/80, matrix=256 x 256, 3mm slice thickness, 4 NEX) was acquired for detection of the tumor. After the selection of single slice in the center of the map imaging, dynamic T1 gradient dual echo sequence (1000 phases, TR/TE/ = 10/2ms/30, temporal resolution = 1.28 sec, FOV = 20cm, slice thickness=5mm, matrix size=128128) was started 10-15 sec before contrast injection and acquired (Fig 8-4).

Fig 8-4. Gyroscan intra T(philips).

Then, imaging magnetic resonance speed to control and experimental group. speed is expression the gradient, it's meaning growth to blood vessel.

III. RESULTS

1. Cell counting of in-vitro

We cultured cell control group and experiment group.

This cell doubling proliferation time is 12 hour. so we added three kind of drug {BA(Betulinic Acid), Ra-12a, SAHA} per 12 hour. Used drug IC_{50} is BA=5.1 ㎍/㎖, Ra-12a=6.2 ㎍/㎖, SAHA=4.15 ㎍/㎖.

To comparing cell population for staining trypan blue, counting cell number about control group and experiment group. Drug added group's population is fewer than control group. The most powerful drug is Ra-12a among three kind of drug. Because Ra-12a group cell population is lowest (Table 1).

	BA	RA-12a	SAHA	saline
10월24일	9.7*10⁴	9.7*10⁴	9.7*10⁴	9.7*10⁴
10월25일	8.4*10⁵	4.16*10⁵	6.56*10⁵	3.84*10⁷
10월26일	1.91*10⁸	2.5*10⁷	3.41*10⁷	8.4*10⁸
10월27일	1.4*10⁹	6.6*10⁸	1.03*10⁹	2.3*10¹⁰
10월28일	4.6*10¹¹	1.2*10¹⁰	2.4*10¹⁰	9*10¹²
10월29일	1.35*10¹³	5.4*10¹¹	9.8*10¹¹	1.04*10¹⁴
10월30일	0.94*10¹⁴	2.71*10¹³	4.01*10¹³	9.72*10¹⁵

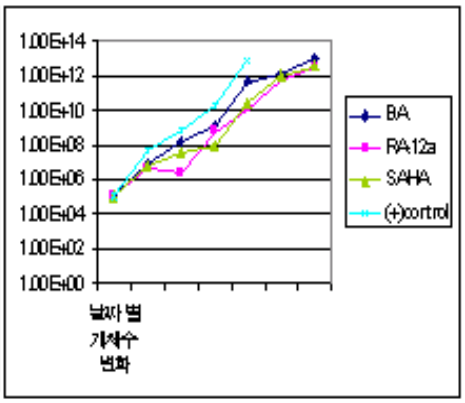


Table 1. Change of cell number to *in-Vitro*

2. Change of tumor size

C3H/HeJ-FasL / 6week. FM3A cells (1.0 X 10^7) were injected in mouse sub-membrane. Beginning to drug injection (1time/2days, total 6~7 times) for becoming complete cancer size (0.7cm x 0.7cm). Three kinds of drug LD_{50} is BA (170 ㎍/㎖), Ra-12A (206 ㎍/㎖), SAHA (138 ㎍/㎖).

When every time process about drug injection, then we measured tumor size. red arrow look like meaning no effect drug. But experiment group tumor is beginning necrosis from central to out (Fig 9).

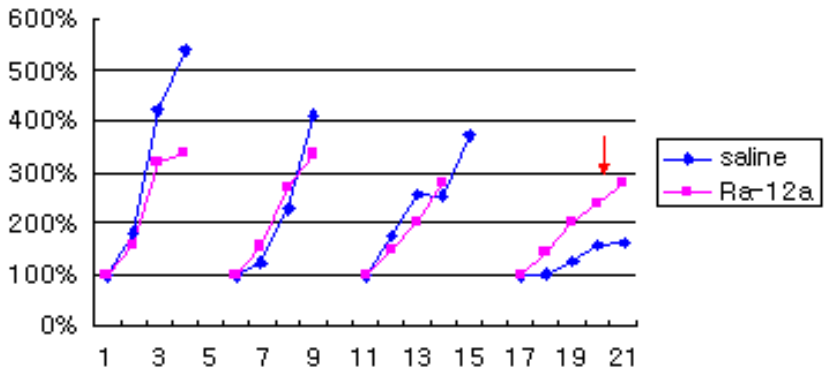


Fig 9. Change of tumor size control and experiment group

3. ELISA for VEGF

Total protein is extracted and tested for VEGF by Quantakine mouse VEGF ELISA according to the manufacturers instructions (R & D Systems, MN) using every total protein is 9.1 ㎍/㎖ (Table 2).

BA	RA-12A	SAL
0.691	0.416	0.545
0.341	0.295	0.572
0.401	0.478	0.511
0.267	0.645	0.483
0.351	0.308	0.444
0.572	0.337	0.481
0.474	0.468	0.517
0.187	0.108	0.152
0.183	0.095	0.178
0.259	0.122	0.239
0.262	0.266	0.421
0.312	0.285	0.412
0.329	0.258	0.39
0.265	0.194	0.298

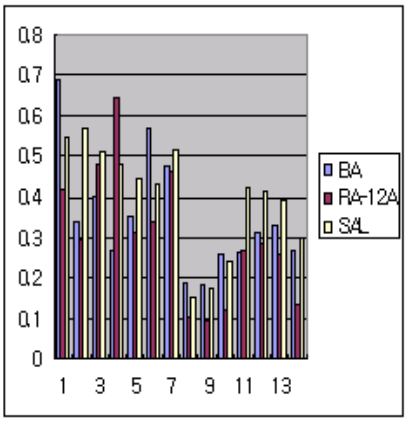


Table 2. Effect of Ra-12a on VEGF gene Expression for ELISA

Vascular endothelial growth factor (VEGF) is analysed by ELISA. we prepared total protein about cancer.

Total protein is extracted and tested for VEGF by quantakine mouse VEGF ELISA according to the manufacturers instructions (R & D Systems, MN).

Results were expressed as ㎍/㎖ of extracted total protein.

Ra-12a group VEGF density is lowest among three group.

So the most powerful drug is Ra-12a.

4. SDS PAGE & western blot analysis

TIMP-2 detection. lane1:marker, lane2,3:BA, lane4,5:ra-12a, lane7,8:SAHA, lane9,10:control (Fig 10, A). 12%-sds gel loading (Fig 10, B).

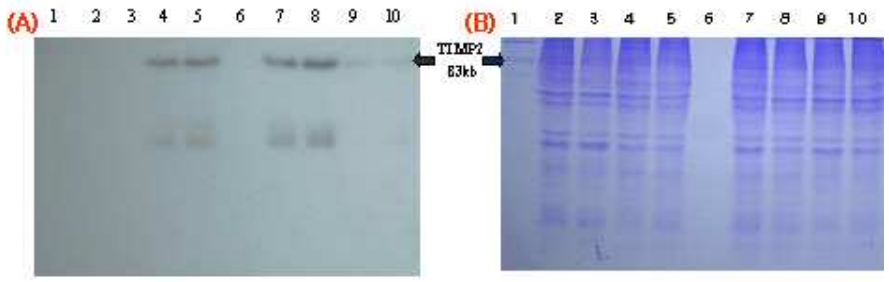


Fig 10. Effect of Ra-12a on TIMP-2 gene Expression for Western blot analysis.

This is western blot analysis use to TIMP2 antibody(fig 10, A). lane 7,8 is total protein detection to TIMP2 from injected Ra-12a drug mouse tumor. compared with other lane, Ra-12a lane is most activation TIMP2. the other hand, Ra-12a is most powerful of drug for activation TIMP2 (Fig 10, A).
Stacking gels were 4.5% polyacrylamide and separating gels were 7.5% polyacrylamide. Paired mini-gels (Mini-protein II cell, Bio-Rad Laboratories, U.S.A.) were loaded with 39.2μg protein per well (Fig 10, B).

5. β-actin detection

Detected TIMP2, lane M: marker, lane1,2:BA, lane3,4:ra-12a, lane6,7:SAHA, lane8,9:control (Fig 11, A)

This band is β-actin detection to prove total protein 39.2 ㎍/4㎖ (9.8 ㎍/㎖) loading. Same is migration of band meaning same as used total protein. (Fig 11, B)

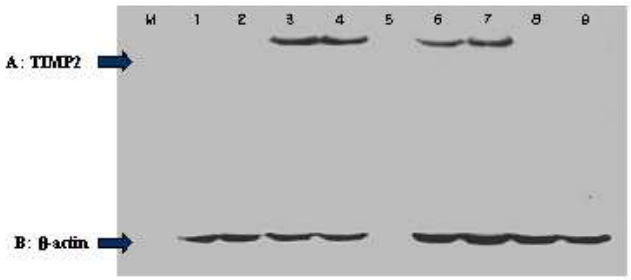


Fig 11. Used same volume total protein to Western blot analysis for β-actin detection

6. Dynamic MR imaging for Control group.

White arrow area is mouse tumor. perform an operation under a general anesthesia, we injected magnetic resonance solution to mouse blood vessel (Fig 12, A).

Measured speed to magnetic resonance of white arrow area (mouse tumor).

Used machine name is gyroscan intra T(philips) (Fig 12, B).

Fig 9, B graph is so upward tendency. it is meaning that blood vessel proliferation is iron out.

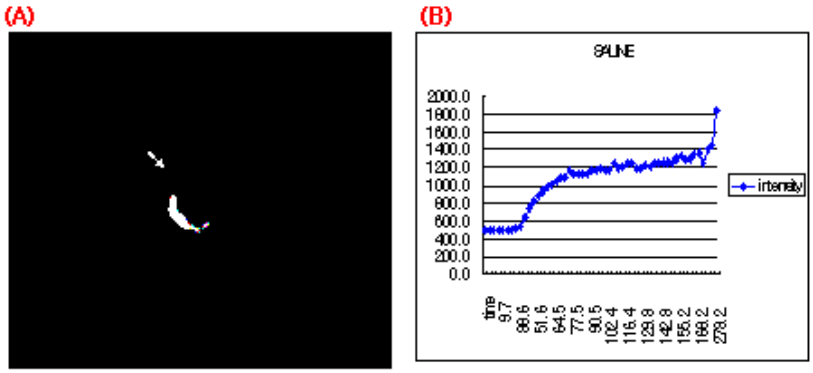


Fig 12. (A) D-MRI for control group. (B):migration speed of magnetic resonance about injection to mouse

7. Dynamic MR imaging for Experimental group

White arrow area is mouse tumor. perform an operation under a general anesthesia, we injected magnetic resonance solution to mouse blood vessel (Fig 13, A).

Measured speed to magnetic resonance of white arrow area (mouse tumor). used machine name is gyroscan intra T (philips) (Fig 13, B).

(B) graph is so gently-sloping. it is meaning that blood vessel proliferation is inhibited to Ra-12a.

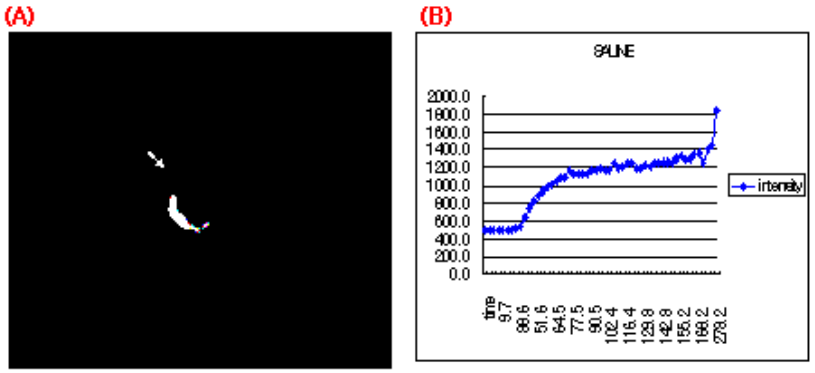


Fig 13. (A): D-MRI for Experimental group. (B):migration speed of magnetic resonance about injection to mouse

Ⅳ.DISCUSSION

1. Histone deacetylase (HDAC) inhibitor is anticancer agents.

Histone deacetylase (HDAC) inhibitors have emerged recently as promising anticancer agents. They arrest cells in the cell cycle and induce differentiation and cell death. The anti-tumor activity of HDAC inhibitors has been linked to their ability to induce gene expression through acetylation of histone and nonhistoneproteins.

2. Ra-12a is HDAC (histone deacetylase) inhibitor.

Ra-12a added group's population is fewer than control group. The other hands, the most powerful drug is Ra-12a among all kind of drug.

3. Ra-12a is anti angiogenic drug.

Ra-12a group VEGF density is lowest among three group. So the most powerful drug is Ra-12a. VEGF, binds to cell-surface VEGF is a more potent EC mitogen than is VEGF In addition, we recently characterized a novel VEGF found on the surface of tumor cells that is specific in that it binds VEGF.

4. D-MRI is non-invasive quantification method.

This VEGF activity was observed to d-MRI non-invasive quantification method. In each tumor the perfusion and vascular permeability were measured by using bolus intravenous injection of 0.2 mmol/kg Gd-DTPA (Magnevist, Schering, Germany) mixed with 0.3ml of saline. Graph is compared about gradient. It is meaning that blood vessel proliferation is inhibited to Ra-12a.

V .CONCLUSION

1.cancer VEGF is regulated by Ra-12a.

Cancer needs some blood vessel and MMPs (matrix metalloproteinases) to proliferation. Angiogenesis is an important step in the development of cancer and is necessary for primary tumor growth, invasiveness, and metastasis.

Vascular endothelial growth factor (VEGF) is believed to be important for the process of initiation of angiogenesis and is a major mediator of cancer angiogenesis. Overexpression of VEGF has been shown in various cancers.

Ra-12a can reduction to VEGF, it repress cancer activity.

So Ra-12a is inhibition drug to the platelet-derived growth factor PDGF/VEGF family of growth factors, is a key regulator of angiogenesis.

2.Cancer TIMP-2 is activated by Ra-12a.

Ra-12a have ability to activation Tissue inhibitors of metalloproteinases (TIMPs). To be activated TIMPs regulate matrix metalloproteinase (MMP) activity controlling the breakdown of extracellular matrix components and, thus, play an important role in the process of invasion and metastasis.

Moreover, there are several new functions, growth control, apoptosis, and anti-angiogenesis, in which TIMPs seem to be

involved. The aim of this study was to elucidate the role of TIMP-2 in human cancer assessing TIMP-2 protein expression in breast cancer evaluating its importance relative to manipulation cancer proliferation.

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Mouse Tumor에서 Ra-12a의 효과를 비관혈적 방법인 D-MRI로 blood vessel activity 의 측정

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최근에 새로운 항암기전으로 관심을 끌고 있는 세포 내 신호전달에 관련된 단백질들의 저해제, 세포주기 관련 저해제, 핵산의 위상학적 변형 저해제 및 신규혈관 형성 (angiogenesis) 저해제들 중 HDAC (histone deacetylase)의 저해제와 angiogenesis의 저해제는 암 치료에 새로운 이정표로써 부각되고 있다.

HDAC의 저해제로써 새로운 약물인 Ra-12a는 anti-angiogenic activity도 함께 나타내는 것으로 실험 결과 밝혀졌다.

특히 Ra-12a HDAC의 저해제로서 뿐만 아니라 angiogenesis의 억제제로서 동시에 작용하기 때문에 최근에 관심을 갖는 분야 중의 하나이다. 그러나 HDAC나 angiogenesis를 억제하는 Ra-12a는 차세대 항암제로서 유망한 것들이지만 아직까지 임상 실험이 완전히 끝나지 않은 상태이고 이들의 유도체들 또한 거의 알려져 있지 않다.

기존 상용화 된 화합물은 fungal metabolite로서 합성에 의해서는 20 step이상의 합성 과정을 거치므로 상업화가 어렵거나 미생물에 의한 발효 및 추출 과정에서는 소량만이 얻어지므로 항암 물질로 사용하기에는 거의 불가능한 실정이다. 그러나 Ra-12a 는 산업화가 용이하며, 짧고 간단한 합성 또는 추출 과정을 가지는 장점이 있다.

cancer가 활성화 되려면 angiogenesis 란 과정을 통해 신생혈관을 형성하여 cancer의 생존에 필요한 물질들을 공급 받는 것으로 알려져 있다. 본 실험에 사용된 약물은 이러한 신생혈관의 생성을 억제함에 따라 cancer의 활성을 억제하는데 그 목적이 있다. 또한 생체내 자연 항암 물질인 TIMPs family 중 cancer의 발현에 관여하는 TIMP2 는 cancer의 활성에 꼭 필요한 MMP를 억제하므로 또한 cancer의 활성을 억제한다.

본 실험에 사용된 동물모델은 C3H/Je라는 학명으로 일본 잭슨사에서 구매하였으며 사용된 cancer cell line은 FM3A라는 breast

cancer cell line 이다. 이 cell을 in-vitro 에서 1x107개를 culture 한 후 mouse의 sub membrane에 injection하게 된다.

완전히 tissue화 될 때까지 sacrifice를 하여 새로운 개체에 transfer 하여 약0.5x0.5(단위:cm)크기가 되었을 때 각 종류별 drug의 LD50값으로 1회/2일 간격으로 피하 주사한다.

이 후 본 실험에 사용된 약물과 control을 비교하기 위하여 VEGF에 관한 ELISA, TIMP2에 관한 western blotting을 수행하고 마지막으로 실험군과 대조군의 혈관생성정도를 비관혈적 측정방법인 D-MRI로 관찰한다.

기존의 증명 방법으로써 널리 쓰이는 방법은 대부분 실험동물의 sacrifice를 전제 했지만 본 논문의 D-MRI 방법은 sacrifice 없이 non-invasive 한 방법으로써 의미가 있다하겠다.

핵심되는 말 :MMPs(matrix metalloproteinases),
TIMP2(Tissue inhibitors of metalloproteinase 2),
VEGF(Vascular endothelial growth factor),
Ra-12a,(New drug),
D-MRI(Dynamic MR imaging)