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Resveratrol regulates CXCR4 expression and downstream signaling of Caki-1 cells *in vivo* and *in vitro*

So-Jung Yoon

Department of Medical Science

The Graduate School, Yonsei University

Resveratrol regulates CXCR4 expression and downstream signaling of Caki-1 cells *in vivo* and *in vitro*

Directed by Professor Sung Joon Hong

The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

So-Jung Yoon

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This certifies that the Master's Thesis of So Jung Yoon is approved.

Thesis Supervisor : Sung Joon Hong

Nam Hoon Cho

Hea Young Oh

The Graduate School
Yonsei University

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Lastly, and most importantly, I wish to thank my parents and my brother. Particular thanks, of course, to Jong -Seok Moon. They have always supported and encouraged me to do my best in all matters of life. To them I dedicate this thesis.

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ABSTRACT

Resveratrol regulate CXCR4 expression and downstream signaling of Caki-1 cells *in vivo* and *in vitro*

So-Jung Yoon

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Sung Joon Hong)

Resveratrol, a phytoestrogen found in food products such as grapes and wine, produces various physiological effects. Resveratrol possesses anticancer activity: it inhibits proliferation and induces apoptotic cell death in multiple cancers cells. The chemokine stromal cell-derived factor-1α (SDF-1α)/CXCR4 axis plays a prominent role in tumorigenesis and promotes angiogenesis and migration of tumor cells to metastatic sites in many cancers. Expression of CXCR4 was shown to be upregulated in most renal cell carcinoma (RCC) patients. In human RCC, CXCR4 plays a major role in the proliferation and growth of cancer cells through activation of PI3K/AKT signaling. We demonstrate that resveratrol suppresses the expression of CXCR4, and inhibits cell proliferation and growth of the human kidney cancer cell line, Caki-1. We show that resveratrol suppresses RNA and protein expression of CXCR4 in Caki-1 cells by a mechanism involving transcriptional regulation. Recombinant SDF-1α, the ligand of

CXCR4, induces cell proliferation and AKT and extracellular signal–regulated kinase(ERK) 1/2 activation, and can, independently, cause renal cell carcinoma cells proliferation. Resveratrol inhibits cell growth and proliferation caused by SDF-1α in Caki-1 cells. Also, we examined the therapeutic efficacy of resveratrol *in vivo* by treating SCID mice bearing Caki-1 cells. Resveratrol suppressed both tumor growth and the expression of CXCR4 in the xenograft model. These data suggest that resveratrol may be a therapeutic agent for kidney cancer as an effective inhibitor of CXCR4. These results may provide a new therapeutic strategy for the use of a natural compound in the treatment of human kidney cancer.

Key Words: CXCR4, resveratrol, kidney cancer

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I. INTRODUCTION

RCC accounts for approximately 3% of new cancer incidences or mortality rate in the Unites States. RCC is a very heterogeneous disease that consists of various subtypes based on genetic and morphological features [1, 2]. A broad classification of RCC includes several histological subtypes: clear cell, papillary, chromophobe, and collecting duct. The clear cell carcinomas are the most prevalent and constitute up to 80-85% of cases. The clear cell tumors exhibit loss of heterozygosity (LOH) and somatic mutation of the Von Hippel-Lindau (VHL) tumor suppressor gene. Because of these features, clear cell tumors have an invasive and metastatic phenotype. At present, surgical resection is the most effective treatment for localized RCC. Despite continuing investigation and increased understanding of the biology of the disease, for the management of advanced or metastatic disease there is a lack of effective adjuvant treatment such as chemotherapy, radiotherapy, or biologic response modifiers. Recently,

small molecule targeting drugs, small-molecule multi-kinase inhibitors, anti-VEGF antibody, and a mammalian target of rapamycin inhibitor have been developed, but responses are mostly partial and of limited efficacy [3].

Previous studies have suggested that chemokines may play a major role in mediating tumor growth and survival [4, 5]. Chemokines are a group of chemoattractant cytokines that mediate several cellular functions. CXC chemokine receptor, fusin, (CXCR4), a seven-membrane spanning G protein–coupled receptor, is expressed in various cells such as T lymphocytes, monocytes, neutrophils, and endothelial cells. It interacts specifically with SDF-1α, and activation of CXCR4 is induced by SDF-1α binding. Activated CXCR4 induces several signaling cascades, including the phosphatidylinostiol 3-kinase and mitogen-activated protein kinase cascades, which induce cytoskeletal rearrangement, anti-apoptosis effects, and cell growth [6]. Signal transduction pathways induced by CXCR4 activation stimulate cell growth and proliferation in cancer cells. Research has shown that CXCR4 is involved in increasing the metastatic potential of RCC, colon, and breast cancer cells. In RCC, it is known that pVHL negatively regulates CXCR4 expression, owing to its capacity to target hypoxia-inducing factor-1α (HIF-1α) for degradation under normoxic conditions. This process is suppressed under hypoxic conditions, resulting in HIF-1α-dependent CXCR4 activation [7]. The activation of HIF-1α and CXCR4 induces the proliferation, growth, and metastatic phenotype of RCC. It has been shown that CXCR4 expression correlates with growth and progression of various cancer cell lines, but the regulatory mechanism of CXCR4 expression is poorly understood.

Resveratrol (3,4',5-trihydroxy-trans-stilbène) is a natural phytoalexin present in grapes and a variety of plants, including peanuts, mulberries, and legumes [8]. It has been shown to inhibit growth of several types of cancer, including breast cancer, prostate cancer, pancreatic cancer, and colon cancer [9]. These studies support the idea that resveratrol possesses anti-cancer activity by interfering with different cellular events associated with initiation, promotion, and progression of multi-stage carcinogenesis. In this report, we investigated whether resveratrol influences CXCR4 expression in human kidney cancer cells *in vitro* and *in vivo*, and also tested whether resveratrol could serve as a potential therapeutic agent or adjuvant in the treatment of human kidney cancer.

II. MATERIALS AND METHODS

Reagents

Resveratrol (>99% pure) was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in absolute ethanol.

Cell culture

The human RCC cell lines, Caki-1 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cell cultures were maintained in McCoy's 5A medium containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA), 100 units/ml penicillin, and 100 mg/ml streptomycin (Invitrogen).

Reverse transcription-PCR analysis

Total RNA was isolated from cultured cells using the TRIzol reagent (Invitrogen) according to the manufacturer's protocol. For quantitative RT-PCR, cDNAs were synthesized from 4 μg of total RNA using oligo primers and SuperScript reverse transcriptase II (Invitrogen) according to the manufacturer's protocol. The resulting cDNA 1 μl was subsequently used in RT-PCR. The DNA was amplified for 25 cycles using CXCR4 primers: CXCR4 5' primer, 5'-TAACTACACCGAGGAAATGGGCTCA-3' and 3' primer, 5'-GTTAGCTGGAGTGAAAACTTGAAGACTCAG-3'; glyceraldehydes-3-phosphate dehydrogenase (GAPDH) 5' primer, 5'-CCCCTTCATTGA-CCTTCAACTA-3' and 3' primer, 5'-

GAGTCCTTCCACGATACCAAAG-3'. As an invariant control, GAPDH was used. Each PCR cycle was carried out for 30 sec at 94 °C, 30 sec at 57.5 °C and 1 min 30 sec at 72 °C. PCR products were then separated electrophoretically in a 1% agarose DNA gel and stained with ethidium bromide.

Immunoblot analysis

Caki-1 cells were seeded in 100mm dish in media containing 10% FBS at a density of 1 x 10^6 cells/dish. Two days later, the cells were incubated with 50 and 100 μM resveratrol for 48 hr. The cells were harvested using diluted Laemmli buffer and then briefly sonicated. Total cell lysates were centrifuged at 12000 rpm for 10min 4 °C. Supernatants were collected and determine protein concentration, using a modified Bradford assay kit. The proteins (40 or 45μg) were separated in 8~10% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred to Protran nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Membranes were blocked in 5% nonfat dry milk solution in Tris-buffered saline containing 0.1% Tween-20 (TBST) for overnight and incubated for 3 hr with the primary antibody diluted in TBST, and then incubated with 1:5,000 diluted solution of ImmunoPure HRP conjugated goat anti-rabbit or anti-mouse IgG antibody (Cat. No. # 31462 or # 31430, Santa Cruz BiotechnologyInc., Santa Cruz, CA) at room temperature for 20 min. The immuno-reactive bands were visualized with the SuperSignal West Pico Chemiluminescent System (Pierce, Rockford, IL). The specific

bands were detected against phosphorylated forms of AKT and ERK and CXCR4, native AKT, PI3K, ERK and β-actin (Santa Cruz).

Recombinant plasmids

The CXCR4 promoter was constructed by sub-cloning from the genomic DNA of Caki-1 cells using the follow primer; 5' primer, 5'-GGCAGCTTATAGAGAACCACCTTGTAA-3' and 3' primer, 5'-TTTAAAATTCACCAAATTCTTTTGCA-3'. The resulting 2kb fragment for the CXCR4 promoter was inserted into the SmaI site of pGL3-basic vector (Promega Corp., Madison, WI) and the plasmid construct was called as the pCXCR4-Luc reporter. The sequences of plasmid were confirmed by DNA sequencing using the RV3 primer 5'-CTAGCAAAATAGGCTGTCCCC-3'.

Transient transfections and reporter gene assays.

Caki-1 cells seeded at 2 x 10^5 cells/well in 6-well plate were cultured with the cell culture medium described above. The next day, the cells were transfected with the indicated plasmid DNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After 6 hr incubation, the medium was changed with 10% FBS containing fresh medium. The next day, each three wells treated with or without 50 and 100 μM resveratrol. After 48 hr incubation, cells were washed with PBS and lysed in 200 μl passive lysis buffer (Promega Corp.). Aliquots of 5 μl of cleared lysates were used to assay luciferase activity, using a luciferase reporter assay kit (Promega Corp.). The values were

normalized with protein contents. Each transfection experiment was performed in triplicate.

MTT assay

The effect of resveratrol on cell proliferation was measured using an MTT-based assay. Briefly, the cells (5×10^3 cells / well) were incubated in triplicate in a 96-well plate in the presence of various concentrations of resveratrol and/or ethanol in a final volume of 0.1 mL for 48 hr. The number of viable cells was counted using CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI) according to the manufacturer's instructions. Thereafter, 0.02 mL of MTT solution was added to each well and then incubated for 4 hr. It recorded the absorbance at 490nm using a 96-well plate reader (Molecular Devices Corp. Sunnyvale, CA). The following formula was used: percentage cell viability = (OD of the experimental samples / OD of the control) x 100

Treatment of Caki 1-derived xenografts

Male SCID mice (6-week-old) (Japan SLC, Hamamatsu, Japan) were maintained in pathogen-free sterile isolators according to institutional guidelines, and all food, water, caging, and bedding were sterilized prior to use. Caki-1 cells (5×10^6 cells) were injected s.c. Tumor volume was determined by measuring length, width, and depth of the tumor every 1 days using a caliper, which was calculated using the equation: tumor volume (cm^3) = 0.523 x [length (cm) x $width^2$ (cm^2)]. At a tumor size of approximately 40 mm^3 the mice were divided into two

subgroups. One group received daily intraperitoneal injections of resveratrol dissolved in ethanol at a dose of 50 mg/kg once every 2 days for a total of 10 injections while the other group received daily injections of vehicle only. Following 3 weeks of treatment, the mice were sacrificed and the tumors fixed in 4% paraformaldehyde, sectioned. Remaining tumor samples were flash frozen under liquid nitrogen and stored at −80°C for resveratrol analysis as described below. All protocols for animal studies were reviewed and approved by the Institutional Animal Care and Use Committee at Yonsei University.

Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). Statistical significance was evaluated with an unpaired Student's t test for comparison between two groups or with analysis of variance for comparison between multiple groups using Prism 4 software (Graphpad Software, San Diego, CA). A probability value of $p<0.05$ was considered significant.

III. RESULTS

Effects of resveratrol on the expression of CXCR4 mRNA and protein in Caki-1 cells

To observe the effect of resveratrol on CXCR4 in human kidney cancer cells, we selected a representative human kidney cancer cell line, Caki-1. In Caki-1 cells, CXCR4 expression was identified at both the mRNA and protein levels. As shown in Fig. 1A, expression of CXCR4 mRNA was suppressed in a dose-dependent manner by resveratrol. Also, we performed immunoblot analysis to confirm altered CXCR4 expression in resveratrol-treated Caki-1cells. Expression of CXCR4 was completely suppressed in the presence of 100 μM resveratrol in Caki-1 cells (Fig. 1B). These experiments demonstrate that resveratrol affects CXCR4 expression in Caki-1 cells. Because mRNA levels of CXCR4 were decreased by resveratrol, we expected that the regulation of CXCR4 expression was dependent on transcriptional levels. In order to identify the regulatory mechanism of CXCR4 expression by resveratrol in Caki-1 cells, we generated a pCXCR4-Luc reporter construct. To observe the transcriptional regulation of CXCR4, we assessed the responsiveness of the CXCR4 promoter by resveratrol in Caki-1 cells (Fig. 1C). CXCR4 promoter activity was gradually decreased by a dose-dependent addition of resveratrol. Resveratol at a dose of 100 μM suppressed CXCR4 promoter activity by 50% in Caki-1 cells. These results indicate that suppression of CXCR4 expression is regulated at the transcriptional level in Caki-1 cells.

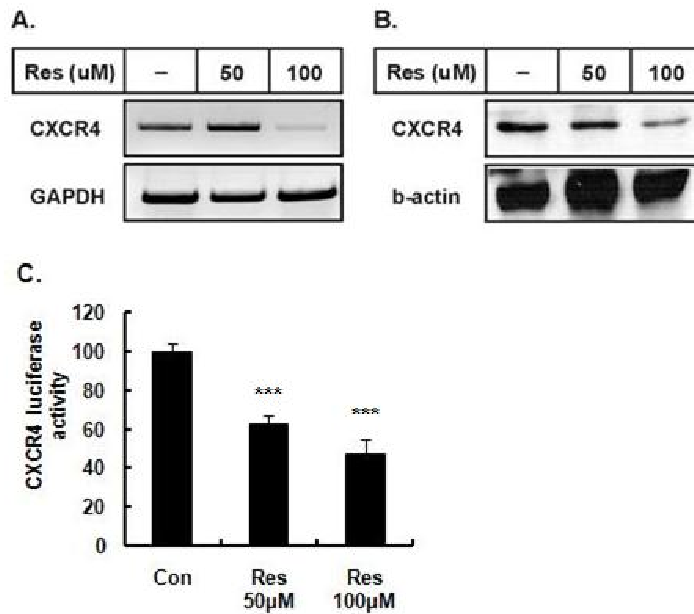

Figure 1. CXCR4 mRNA and protein expression in Caki-1 cells in response to resveratrol. (A) Agarose gel (1%) stained with ethidium bromide showing the amplified RT-PCR product with equal amounts of RNA. GAPDH was used as the internal control. Total RNA isolated from Caki-1 cells treated with resveratrol for 24 hr. (B) Caki-1 cells were treated for 48 hr with resveratrol and CXCR4 expression was determined by western blot analysis. (C) Caki-1 cells were transfected with pCXCR4-Luc reporter construct. After 6 hr transfection, the medium was changed using 10% FBS containing fresh medium. The next day, each three wells treated with or without 50 or 100 μM resveratrol. The luciferase activities were measured at 48 hr with resveratrol. Each transfection experiment was performed in triplicate. ***, $p < 0.005$ versus control value.

AKT and ERK-signaling through CXCR4 are inhibited by resveratrol in Caki-1 cells

To study the intracellular pathways involved in renal cell carcinoma survival, we focused on ERK1/2 activation, which converts extracellular stimuli to intracellular signals that control gene expression, cell proliferation, and survival, through the SDF-1α/CXCR4 axis [10]. Another important signal transduction pathway in renal cell carcinoma is the PI3K/AKT pathway. We were interested in whether resveratrol could inactivate the ERK1/2 and AKT pathways in Caki-1 cells. We pretreated Caki-1 cells with resveratrol for 48hr and analyzed AKT and ERK1/2 activation by western blot. Our results showed that resveratrol inhibited the expression of phosphorylated ERK1/2 as compared to the slight activation of ERK1/2 detected under basal conditions. AKT activation also occurred under basal conditions and was further decreased after resveratrol stimulation (Fig. 2A). We then assayed by MTT to directly determine the effect of resveratrol on the viability in Caki-1 cells. As shown in Fig. 2B, the viability and proliferation of Caki-1 cells were decreased 60% compare to controls when treated with resveratrol at concentrations of up to 50 μM. The effects of resveratrol may be exerted through modulation of CXCR4 gene transcription and rapid signaling by cell surface receptor crosstalk.

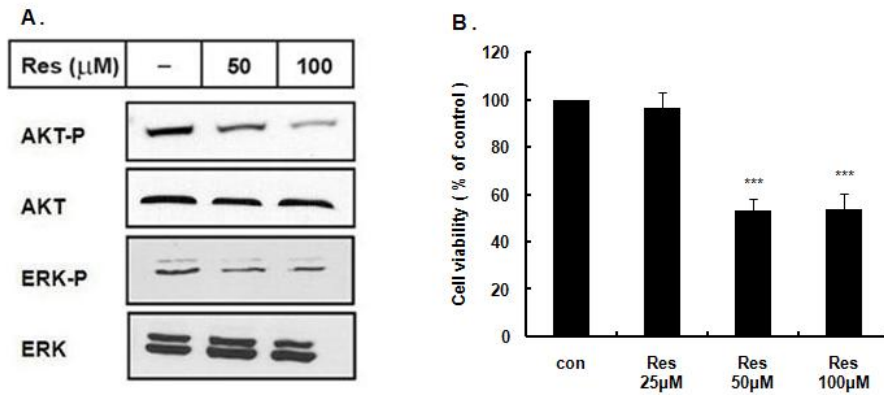

Figure 2. The effect of resveratrol on AKT and ERK activity and cell survival in Caki-1 cells (A) Western blot analysis of phosphorylated and total forms of ERK(p44/p42) or AKT (p60) in Caki-1 cells that were either untreated or treated-resveratrol. (B) The cell viability was measured by the MTT assay. Cells were cultured for 24 hr in the absence or presence of resveratrol (25, 50, and 100 μM) for 48 hr and then assayed. Data are expressed as absorbance at 490 nm. ***, $p < 0.005$ versus control value.

SDF-1α effects on downstream signaling and cell survival in Caki-1 Cells

To investigate whether SDF-1α affects downstream signaling and cell survival in Caki-1 cells, Caki-1 cells were maintained in serum free growth conditions for 24 hr, then treated with SDF-1α for various amounts of time. We found that Caki-1 cells showed peak pERK activity after 5 min and diminished over 30 min (Fig. 3A). The peak of pAKT activity also occured after 1min and decreased slightly 10 min later. We then assayed whether SDF-1α influenced the proliferation of Caki-1 cells by MTT. As shown in Fig. 3B, the viability and proliferation of Caki-1 cells were increased 50% when treated with SDF-1α for 2 days compare to control cells.

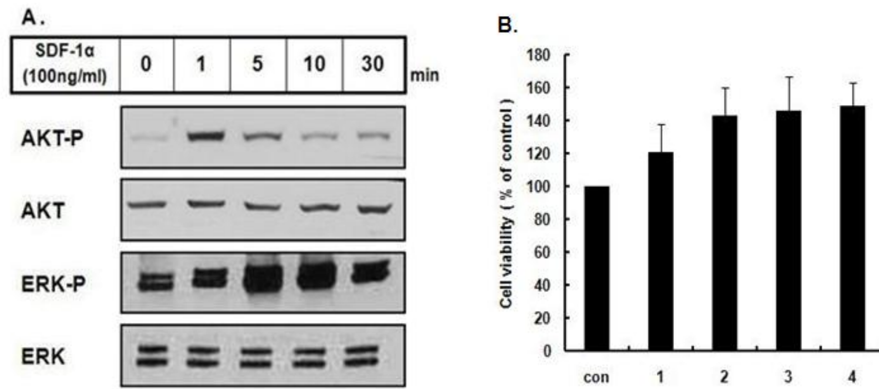

Figure 3. Activation AKT and ERK by SDF-1α and its effects on cell survival (A) Caki-1 cells were treated with 100 ng/mL SDF-1α for 0, 1, 5, 10, and 30 min. The cell lysates were prepared and used for western blot analysis with phospho-AKT, phospho-ERK, total AKT or ERK antibody to investigate AKT and ERK activation. (B) Cells were stimulated with SDF-1α for different periods (100 ng/ml). Data are expressed as absorbance at OD 490 nm. Data shown are representative of a minimum of three independent experiments.

Resveratrol modifies SDF-1α activity of Caki-1 cells

To investigate whether resveratrol could affect the functional responsiveness for SDF-1α in Caki-1 cells, we treated cells with resveratrol before SDF-1α stimulation. Caki-1 cells were maintained in serum free growth conditions for 24 hr then treated with or without resveratrol for 24 hr before stimulation of SDF-1α. In contrast to stimulation of SDF-1α (Fig. 4A), the activity of AKT and ERK was strongly diminished as compared to the control group. Caki-1 cells were also maintained in medium with or without resveratrol for 24 hr, and 100 ng/mL of SDF-1α was added for 24 hr. Pre-treatment with resveratrol followed by SDF-1α stimulation resulted in OD490 nm values lower than that when cells were not pretreated with resveratrol (Fig. 4B). Resveratrol suppressed SDF-1α-mediated proliferation of Caki-1 cells in a dose-dependent manner.

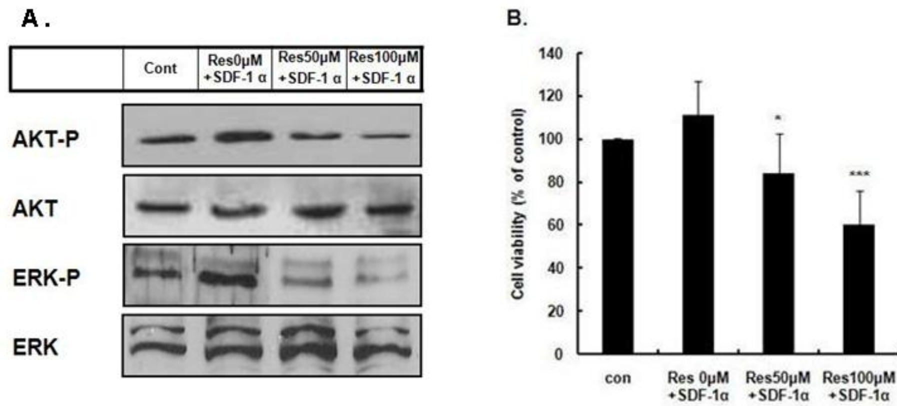

Figure 4. Resveratrol inhibits AKT and ERK activation induced by SDF-1α and cell survival. (A) Caki-1 cells were pretreated with 100ng/mL resveratrol (50 or 100 μM) for 24 hr before their stimulation with SDF-1α for 5 min. The cell lysates were prepared and used for western blot analysis with phospho-AKT and ERK antibody to investigate AKT and ERK activation. (B) The cell viability was measured by the MTT assay. Data are expressed as absorbance at 490 nm. *, $p < 0.01$ and ***, $p < 0.005$, versus unstimulated cells or versus SDF-1α-treated cells in the absence of resveratrol, respectively.

Resveratrol inhibits tumor growth and CXCR4 expression *in vivo*

Because we had shown that resveratrol blocked Caki-1 cell growth through the SDF-1α/CXCR4 axis in culture, we further examined the therapeutic efficacy of resveratrol *in vivo* by treating SCID mice bearing human RCC Caki-1 tumor xenografts. As shown in Figure 5A, tumor volume increased in both groups. After 28 days, the mean volume of the Caki-1 tumor in mice treated with resveratrol at 50 mg/kg was 120 mm^3, whereas that of mice treated with vehicle was 440 mm^3. Indeed, there was a significant delay in tumor growth in animals treated with resveratrol at 50 mg/kg ($p < 0.0001$). Tumors were then harvested from the two groups of mice and were processed for western blot analysis for histology. To confirm that resveratrol blocked the PI3K/AKT and ERK pathways *in vivo*, AKT and ERK phosphorylation status were measured by western blot. The results show that, as *in vitro* in cultured cells, AKT and ERK are present in an inactive form in RCC tumors *in vivo*. Immunoblot analysis clearly showed reduction in phosphorylated AKT and ERK levels in tumors of resveratrol (50 mg/kg)-treated mice as compared to tumors of control-treated mice. (Fig. 5B) Treatment with resveratrol seems to act on the inhibition of CXCR4 expression, resulting in inhibition of the CXCR4-mediated signal pathway.

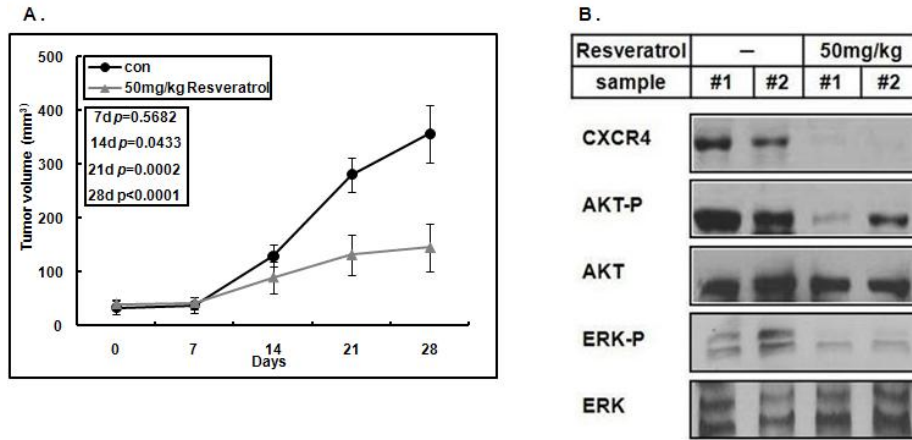

Figure 5. Inhibition of tumor growth and CXCR4 expression after resveratrol treatment in Caki-1 cell xenograft model. Caki-1cell tumor xenografts were established in nude mice (n = 10). The linear coefficients and standard errors from the random effects model are shown in the graph. (A) Comparison of tumor volumes between animals, resveratrol-treated (n = 6) and untreated (n = 4) groups. (B) Distribution of CXCR4 and its target gene products in xenograft tumors. Western blot analysis was performed for with CXCR4, phospho-AKT, AKT, phospho-ERK1/2, and ERK in whole fractions from xenograft tumors with resveratrol-treated or untreated mice.

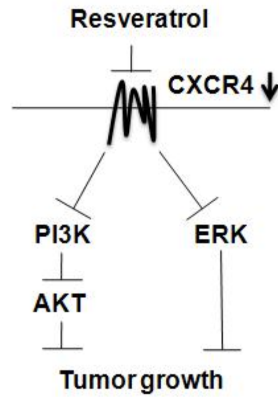

Figure6. Resveratrol suppresses tumor growth through the inhibition of CXCR4 expression. This diagram was based on this and other studies. The model suggests that resveratrol downregulates CXCR4 expression. Accordingly, it can also inhibit CXCR4-mediated downstream signaling and corresponding phenomena such as tumor growth.

IV. DISCUSSION

Our study showed that resveratrol effectively inhibits *in vitro* cell proliferation and *in vivo* tumor growth of Caki-1 cells by suppressing the expression of CXCR4. In addition, resveratrol directly affects AKT and ERK phosphoryation, and abrogates SDF-1α/CXCR4 mediated AKT and ERK signaling. These results indicate that resveratrol exerts anti-tumor effects through CXCR4 downregulation.

Resveratrol, a phytoestrogen found in food products such as grapes and wine, causes various physiological effects. Resveratrol has been known as a promising candidate for its chemopreventive effect. Resveratrol possesses anticancer activity and inhibits proliferation and induces apoptotic cell death in multiple cancers cell types *in vitro* [11, 12]. The inhibition of cell cycle and induction of apoptosis are other essential features of resveratrol in various cell lines [13-15].

A complex network of chemokines and receptors exists in the tumor microenvironment [5]. Chemokines exert a variety of biological functions, including regulation of angiogenesis, activation of cell migration, growth promoting effects, and inhibition of apoptosis [6, 16]. The expression of chemokines and their receptors provides an advantage in tumor cells and may supply them with an enhanced ability to proliferate and disseminate. SDF-1α, the ligand for CXCR4, plays a major role in tumorigenesis, including tumor growth, invasion, and metastasis [17, 18]. The SDF-1α/CXCR4 axis mediates multiple signal transduction pathways and a variety of cellular functions, such as cell migration, proliferation, and survival. Sourbier *et al.* showed that SDF-1α/CXCR4 is involved in the PI3K/AKT signaling pathway,

which is known to be constitutively active in kidney cancer and represents a major target in the prevention of defects in cell growth, survival, and motility [19]. We observed that PI3K/AKT and ERK signaling is important for SDF-1α/CXCR4-mediated cell growth and proliferation in Caki-1 cells, and resveratrol suppresses SDF-1α/CXCR4 signaling though a decrease in CXCR4 expression in these cells. Our results suggest that resveratrol, as a CXCR4 inhibitor, may be effective in inhibiting the growth and proliferation of RCC cells.

Advanced kidney cancer indicates a more metastatic phenotype than other cancers. Because kidney cancer cells have a mutation or loss of function of the VHL tumor suppressor gene, the growth and invasion of kidney cancer depends on hypoxic conditions. Several studies have suggested that HIF-1α regulates the expression of CXCR4 in RCC. These studies further suggest that the loss or functional inactivation of the protein product of VHL results in persistent activation of HIF-1α and a dramatic increase in CXCR4 [20, 21]. Thus, elevated CXCR4 expression has been detected in several human RCC cell lines and tumor samples, while only minimal CXCR4 expression has been detected in normal kidney tissues [18, 22]. Some studied also demonstrated that hypoxia, leading to increased HIF-1α, is critical for the regulation of the expression of CXCR4, and may represent a general scheme in tumor metastasis[23]. Thus, the expression of CXCR4 is activated in a tumor environment by HIF-1α, and the hypoxia-induced CXCR4 activates the PI3K/AKT and ERK signaling pathways in kidney cancer. The growth and proliferation of kidney cancer cells may be more dependent on CXCR4 signaling in hypoxic condition. Therefore, resveratrol may be more effective in

suppressing the growth and proliferation of kidney cancer cells than other cancer cells. In our Caki-1cell tumor xenografts, the expression of CXCR4 is completely suppressed by resveratrol. Also, CXCR4 mediated PI3K/AKT and ERK signaling are suppressed by resveratrol. These results demonstrate that CXCR4 inhibition by resveratrol is effective *in vivo* and resveratrol may be a chemotherapeutic agent, as a CXCR4 inhibitor in kidney cancer.

In conclusion, our studies demonstrate for the first time the importance of resveratrol and its effect on CXCR4-expressing RCC cells. Resveratrol effectively inhibited *in vitro* cell proliferation and *in vivo* tumor growth of Caki-1 cells. In addition, resveratrol directly affected AKT and ERK phosphorylation, and abrogated SDF-1/CXCR4-mediated AKT and ERK signaling. However, it remains to be determined whether AKT and ERK activity are direct targets of resveratrol, diminished via CXCR4 signaling. Further studies are needed using small molecule antagonists for CXCR4, such as AMD3100, and RNA interference, in RCC to understand the effects of resveratrol on CXCR4-mediated signaling.

These results indicate that resveratrol can improve its anti-tumor effect through CXCR4 downregulation. Therefore, we suggest that resveratrol can be a potential therapeutic agent or adjuvant in the treatment of human kidney cancer.

V. CONCLUSION

1. Effects of resveratrol on the expression of CXCR4 mRNA and protein

2. AKT and ERK-signaling through the CXCR4 are inhibited by resveratrol in Caki-1 cells.

3. SDF-1α affects on downstream signaling and cell survival in Caki-1 Cells.

4. Resveratrol modifies the SDF-1α activity of Caki-1 cells.

5. Resveratrol inhibits tumor growth and CXCR4 expression *in vivo*.

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ABSTRACT (IN KOREAN)

신장 암세포와 동물모델에서 Resveratrol에 의한 CXCR4의 발현 및 downstream signaling의 활성 억제

지도교수 홍 성 준

연세대학교 대학원 의과학과

윤 소 정

Chemokine stromal cell-derived factor-1/CXCR4 axis 은 종양형성에 중요하고 혈관 신 생성 및 전이부위로의 암세포 이동 유도, 세포사멸 억제하는 등 다양한 생물학적 역할을 한다. 정상의 신장조직과 비교하였을 때 대부분의 RCC 내 CXCR4의 발현이 높게 나타났다. 또한 여러 암세포에서 SDF-1/CXCR4 axis을 통한 PI3K/AKT 와 ERK signaling 의 활성으로 암세포의 성장과 분열이 확인되었다.

Resveratrol 은 포도, 와인 및 다양한 식물에서 발견되는 phytoestrogen 으로써, 암의 생성 3 단계(initiation, promotion, progression) 에 모두 작용하여 암의 성장을 방해 및 억제하는 것으로 다양한 암세포와 이식 동물모델에서 밝혀지고 있다.

이에 본 연구는 신장 암 세포의 하나인 Caki-1 cell 과 동물 모델을 이용하여 resveratrol 에 의한 CXCR4 발현 및 downstream signaling 의 활성 억제를 확인하였다. 신장암세포

중의 하나인 Caki-1 cells 에서 resveratrol 에 의한 CXCR4 의 발현 조절은 transcriptional level 에서 일어나는 것을 확인하였고 mRNA 와 단백질 발현 모두 감소하였다. CXCR4 의 ligand 인 recombinant SDF-1α 에 의한 AKT 와 ERK signaling 의 활성과 그에 따른 세포 분열의 유도는 resveratrol 에 의해서 억제됨을 알 수 있었다. 또한 Caki-1 cell 로 암이 유도된 동물모델에 resveratrol 50mg/kg 을 3 주 동안 피하주사 하여 resveratrol 의 치료상 효과를 확인하였다. 비교군과 실험군을 비교하였을 때, 암의 성장이 억제되었고 암 조직 내 CXCR4 의 발현도 감소하였다.

본 연구를 통해 resveratrol 은 CXCR4 의 발현을 감소시킴으로써 신장 암을 위한 예방 및 치료 보조제로써 효과적인 역할을 할 것으로 기대된다.

핵심 되는 말: CXCR4, resveratrol, 신장 암