치의학박사 학위논문

FXYD3 knockdown by small interfering RNA inhibits cell proliferation and migration of oral squamous cell carcinoma cells

FXYD3 발현 저하 유도가 구강편평세포암종에서 세포 증식과 이동 능력 억제 효과에 관한 연구

2015년 2월

서울대학교 대학원

치의학과 구강병리학 전공

조 병 찬

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지도교수 홍 삼 표

이 논문을 치의학 박사 학위논문으로 제출함

2014년 11월

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치의학과 구강병리학 전공

조 병 찬

조병찬의 박사학위논문을 인준함

2014년 12월

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ABSTRCT

FXYD3 knockdown by small interfering RNA inhibits cell proliferation and migration of oral squamous cell carcinoma cells

Byoung-Chan Jo

Department of Oral pathology, Graduate School, Seoul National University

(Directed by Professor **Sam-Pyo Hong**, D.D.S., M.S.D., Ph.D.)

BACKGROUND: Oral squamous cell carcinoma (OSCC), the most common malignant neoplasm in oral cavity, is a significant worldwide public health threat. FXYD3 was reported to be overexpressed in prostate, breast, and pancreatic cancers, suggesting its role of carcinogenesis by providing a growth advantage, but downregulated in lung cancers comparing to the normal tissue. However, there have been no reports on the expression and role of FXYD3 in oral squamous cell carcinoma.

METHODS: We generated two FXYD3-knockdown clones (SCC-4si and HO-1-U-1si) from the SCC-4 and HO-1-U-1 OSCC cell lines by transfection with small interfering RNA for FXYD3. Cell proliferation and *in vitro* migration assays were used to investigate the effect of FXYD3 downregulation on cell proliferation and migratory ability in SCC-4si and HO-1-U-1si. Immunohistochemistry was performed to evaluate

the correlation between FXYD3 expression and proliferation in 60 OSCC tissue samples.

RESULTS: The FXYD3-knockdown cells grew significantly slower than the control cells. SCC-4si cells proliferated at 35.7%, 60.9% and 62.7% of the rate of control cells at 24, 48, and 72 hours after transfection, respectively ($P<0.05$). Also, HO-1-U-1si cells did at 47.7%, 28.3% and 34.5%, respectively ($P<0.05$). FXYD3-knockdown cells showed reduced migratory ability. The migration percentages of SCC-4si cells were 37.2% and 38.4% after 24 and 48h, respectively, compared with the control cells ($P<0.05$). Also, those of HO-1-U-1si cells were 33.3% and 44.1%, respectively ($P<0.05$). OSCC showed significantly higher FXYD3 expression than normal mucosa ($P<0.05$). The group with strong expression of FXYD3 had significantly higher Ki-67 labelling index than that with weak expression in OSCC tissue samples ($P<0.05$).

CONCLUSION: These results suggest that the downregulation of FXYD3 induces anti-proliferative and anti-migratory effects in OSCC and that FXYD3 might be a useful target molecule for the treatment of OSCC.

Keywords : oral squamous cell carcinoma, FXYD3, siRNA, proliferation, migration

Student Number : 2009-30653

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I. Introduction

Oral squamous cell carcinoma (OSCC), the most common malignant neoplasm in oral cavity, is a significant worldwide public health threat [1]. The management of OSCC varies considerably, but standard procedure includes surgery with or without neck dissection, followed by adjuvant radiotherapy [2]. Despite all efforts and therapeutic developments, the 5-year survival rate for head and neck cancers has not remarkably improved over the last two decades [1, 3]. In addition, a high percentage of patients have a poor response to therapy and high recurrence rates [4]. Therefore, there has been much interest in the development of novel molecular targets for treatment of OSCC.

The FXYD protein family is a small membrane proteins that have a molecular mass between 7.5 and 24kDa, and all members of the family share a signature sequence of six highly conserved amino acids comprising the FXYD motif (Phe-Xaa-Tyr-Asp) in the NH2-terminus, and two glycines and one serin residue in the transmembrane domain [5]. Mammary tumor 8 kDa (MAT8)/FXYD domain-containing ion transport regulator 3 (FXYD3) was first identified in murine breast cancer induced by the *neu* and *ras* oncogenes, and involved in the regulation of chloride conductance [6]. Recent studies showed that FXYD3 was overexpressed in several types of malignant tumors such as prostate, breast, pancreatic cancers, suggesting that it induces carcinogenesis by providing a growth advantage [7, 8, 9]. Contrary to the reports, FXYD3 seems to be downregulated in lung cancer comparing to the normal tissue [10]. Thus, FXYD3 could play diverse roles in different instances and its significance in carcinogenesis could vary in type of cancer.

However, to our knowledge, there have been no reports on the expression and biological role of FXYD3 in oral squamous cell carcinoma. Therefore, in the present study, we investigated the expression of FXYD3 in OSCC cell lines and tissue samples, effects on cell proliferation and migration of FXYD3-downregulated OSCC cells, and the correlation between FXYD3 expression and proliferation in OSCC tissue samples.

II. Materials and Methods

Cell cultures

SCC-4, SCC-9, SCC-15, SCC-25, HSC-2, HSC-3, HSC-4, and HO-1-U-1 human OSCC cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/ml penicillin and 100 g/ml streptomycin). SCC-4, SCC-9, SCC-15, and SCC-25 were purchased from American Tissue Cell Collection (Manassas, VA, USA). HSC-2, HSC-3, HSC-4, and HO-1-U-1 cells were kindly gifted from Prof. Takata (Hiroshima University, Hiroshima, Japan).

siRNA transfection

A siRNA for FXYD3 (siFXYD3; 5'-CGCCAATGACCTAGAAGATAA-3') and non-silencing control (NSC; 5'-AATTCTCCGAACGTGTCACGT-3') were purchased from Qiagen (Hilden, Germany). SCC-4 and HO-1-U-1 OSCC cells were transfected using the Hiperfect transfection reagent (Qiagen) following the manufacturer's instructions. Briefly, 1 x 10^6 cells were seeded in 6-well plate (SPL, Seoul, Korea) in complete cell culture medium. After overnight incubation, the cells were transfected with siFXYD3 or NSC at a concentration of 50nM, using Hiperfect transfection reagent (6 $\mu\ell$/ml). Transfected cells were named to SCC-4si and HO-1-U-1si, respectively.

RNA preparation and semi-quantitative RT-PCR

The mRNA was purified from the cells using Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's recommended protocol. Two micrograms of RNA was converted into cDNA using random primers and reverse transcriptase (GeneDePot, CA, USA). cDNA was PCR amplified in 30 cycles each consisting of 94℃ for 30 s, 60℃ for 50 s, 72℃ for 50 s.

The primer pairs for FXYD3 and GAPDH were as follows ; FXYD3 forward, 5'- CCG GTC GCG AGA CTG TAC GTC TT–3' ; FXYD3 reverse, 5'–CTG GGG ACA GAG AAC GGT CCT CC-3'; GAPDH forward, 5'- GAA GGT GAA GGT CGG AGT C-3'; GAPDH reverse, 5'- CAA AGT TGT CAT GGA TGA CC -3'. PCR products were electrophoresed on 1.5% agarose gel containing ethidium bromide and visualized by UV-induced fluorescence.

Immunoblotting

Briefly, 70-80% confluent cells were homogenized with 1 ml of lysis buffer (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.2 mM PMSF) and incubated on ice. To the homogenates was added 125 μl of 10% NP-40 solution, and the mixture was then centrifuged for 30 sec at 12,000×g. Supernatant protein was normalized. Fifty micrograms of protein was size-fractionated through a 15% SDS-PAGE gel, transferred to PDVF membrane, and immunoblotted with the anti-FXYD3 (A-8; Santa

Cruz, USA) and anti-α tubulin (B-5-1-2; Santa Cruz) mouse monoclonal antibodies.

Cell proliferation assay

To determine the proliferation rate, cells were seeded on 12-well plates (BD Falcon, Franklin Lakes, NJ, USA) at 3 x 10^4 cells per well in serum-free media. After overnight of incubation, the cells were transfected with siFXYD3 or NSC. And then, after 24, 48, and 72 h, the cells were trypsinized and stained with 0.4% Trypan Blue (Gibco, USA). The total cell number and the proportion of dead cells were counted by hemocytometer. Cell death was determined by the presence of cytoplasmic staining. This assay was performed in triplicate in each experiment, and each experiment was repeated three times.

In vitro migration assay

10^5 cells were placed in the upper compartment (8 μm pore size) of a cell culture insert (BD Falcon). After overnight incubation, cells were transfected with siFXYD3 or NSC. Conditioned medium was added to the lower compartment. Then, after 24 and 48h, the cells on the upper surface of the filter were wiped off with a cotton swab, and the remaining cells were stained with the Diff-Quick stain set (Sysmex, Kobe, Japan). Using a microscope at x 100 magnification, migration was quantified by counting the number of cells that migrated through the pores to the lower side of the filter. This assay was performed in triplicate in each experiment, and each experiment was repeated 3 times.

Tissue samples and immunohistochemistry

Tissue samples of 60 OSCCs were retrieved from the Oral Pathology Registry of Seoul National University Dental Hospital from 2002 to 2006. Tissues were fixed in 10% buffered-formalin and embedded in paraffin. Immunohistochemistry was performed on 4 μm paraffin sections mounted on silicon-coated glass slides, using streptavidin-biotin peroxidase technique as previous described [11, 12]. An anti-human FXYD3 mouse monoclonal antibody (C-20; diluted 1:200, Santa Cruz) and anti-human Ki-67 mouse monoclonal antibody (MIB-1; diluted 1:100, Dako) were used. Staining at the cytoplasm or cell membrane was considered as FXYD-3 positive. The intensity of the staining was graded as negative (no positive cells), weak (< 20% positive cells), moderate (20–50% positive cells) and strong positive (>50% positive cells). In statistical analysis, we considered negative, weak and moderate staining as weak group, and strong staining as strong group [13]. Available 17 cases of normal mucosa within the tissue samples were evaluated. For Ki-67, nuclei from over 1000 tumor cells were counted in the five to eight random fields chosen for each sample and the count was expressed as the percentage of Ki-67 positive cells (Ki-67 labelling index; LI). All procedures followed in this study were in accordance with the guidelines of the Institutional Review Board of the Seoul National University Dental Hospital.

Statistical analysis

Statistical significance was assessed by comparing mean (±SD) values with Student's *t*-

test for independent groups or χ^2 test. P <0.05 was considered statistically significant.

III. Results

FXYD3 expression and screening of OSCC Cell Lines

Of 8 OSCC cell lines, all of those except HSC-4 showed clear expression of FXYD3 mRNA in RT-PCR analysis. And in Western blot, the prominent expression of FXYD3 protein was detected in SCC-4, SCC-15, HSC-4 and HO-1-U-1 (Fig. 1). Considering the above results, SCC-4 and HO-1-U-1 cell lines were chosen for this study.

Confirmation of FXYD3 knockdown by siRNA

SCC-4 and HO-1-U-1 cells were transfected with siFXYD3 or NSC. Therefore, we assayed for siRNA-mediated FXYD3 knockdown by RT-PCR analysis, and we found FXYD3 downregulation in SCC-4si and HO-1-U-1si cells (Fig. 2A). By western blot analysis, FXYD3 was also downregulated in SCC-4si and HO-1-U-1si cells until 72h after transfection (Fig. 3B). Therefore, FXYD3 was knocked down at both the mRNA and protein levels in SCC-4si and HO-1-U-1si cells

Anti-proliferative effects of FXYD3 down-regulation

From the cell proliferation assay, we found that the FXYD3-knockdown cells grew significantly slower than the NSC-transfected control cells (Fig. 3). SCC-4si cells proliferated at 35.7%, 60.9% and 62.7% of the rate of control cells at 24, 48, and 72 hours after transfection, respectively ($P<0.05$). Also, HO-1-U-1si cells did at 47.7%, 28.3% and

34.5%, respectively ($P<0.05$). These data indicated that the down-regulation of FXYD3 has an anti-proliferative effect on OSCC cells.

Reduced migratory ability by FXYD3 down-regulation

FXYD3-knockdown cells showed reduced migratory ability (Fig. 4). The migration percentages of SCC-4si cells were 37.2% and 38.4% after 24 and 48h, respectively, compared with the NSC-transfected control cells ($P<0.05$). Also, those of HO-1-U-1si cells were 33.3% and 44.1%, respectively ($P<0.05$). These data indicate that downregulation of FXYD3 has an anti-migratory effect.

FXYD3 expression and Ki-67 LI in OSCC Tissues

We examined FXYD3 expression and Ki-67 LI in the biopsy specimens from 60 patients with OSCC. FXYD3 protein was detected in the cytoplasm and/or the cell membrane and Ki-67 was detected in the nucleus by immunohistochemistry (Fig. 5). Of 60 OSCC cases, twenty-seven (45.0%) expressed FXYD3 strongly, 14 (23.3%) moderately, fifteen (25.0%) faintly, and 4 (6.7%) not at all. Of 17 normal mucosa cases, eleven (64.7%) expressed FXYD3 faintly, and 6 (35.3%) negatively. OSCC showed significantly higher FXYD3 expression than normal mucosa ($P<0.05$). Ki-67 LI was 54.43 ± 15.83 in FXYD3-strong group and 35.36 ± 14.67 in FXYD3-weak group. There was significant correlation between FXYD3 expression and Ki-67 LI in OSCC ($P<0.05$).

IV. Discussion

In the present study, we induced FXYD3 downregulation by siRNA in SCC-4 and HO-1-U-1 OSCC cells and generated FXYD3 knockdown cells, SCC-4si and HO-1-U-1si. We then investigated the effect of FXYD3 downregulation on cell proliferation and migration. Downregulation of FXYD3 induced anti-proliferative and anti-migratory effects in these OSCC cells. To our knowledge, this is the first description of siRNA-mediated FXYD3 knockdown in oral cancer cells.

The FXYD proteins constitute a family of conserved auxiliary subunits of the Na, K-ATPase and have been the study focus in biomedicine field recently due to their ability to finely regulated the activity of the enzyme complex in various physiological and pathological settings [14]. In mammals, the FXYD family contains seven members including phospholemman (FXYD1) [15], the Na,K-ATPase γ-subunits (FXYD2) [16], mammary tumor marker 8 (Mat-8) (FXYD3) [6], corticosteroid hormone-induced factor, CHIF (FXYD4) [17], protein related to ion channel, Ric (FXYD5) [18], phophohippolin (FXYD6) [19], and FXYD7 [20]. These family members have a molecular mass between 7.5 and 24kDa. They share a common signature of 6 amino acid residues, comprising the FXYD motif, from which the family takes its name, 2 glycine residues and a serine residue. Among them, FXYD3 is able to associate with and modify the transport proteins of Na-K-ATPase, and to interact with Na-K-ATPase as a chloride

channel or a chloride channel regulator.

Studies on the expression of FXYD3 in cancer have mainly been shown that its overexpression was detected during carcinogenesis. In addition to the studies in breast, prostate, and pancreatic cancers [6, 7, 8], Zhang *et al.* [21] reported that FXYD3 protein is highly expressed in majority of urothelial carcinoma of the upper genitourinary tract and bladder compared to normal kidney and bladder tissues. It was known that FXYD3 was highly expressed in colorectal cancers compared to adjacent normal mucosa [22]. Also, Zhu *et al.* [23] presented that overexpression of FXYD-3 is involved in the tumorigenesis and development of esophageal cancers. In agreement with these, our study showed FXYD3 was highly expressed in OSCC compared to adjacent normal mucosa. In contrast to our results, Okudela *et al.* [10] reported that FXYD3 mRNA and protein levels were lower in most of the lung cancer cell lines than in either the noncancerous lung tissue or airway epithelial cell and protein levels were also lower in a considerable proportion of primary lung cancers than in nontumoral airway epithelia. This inconsistency could result from diverse roles of FXYD3 according to type of cancer.

siRNA-mediated downregulation of FXYD3 expression in human prostate cancer cells leads to a significant decrease in cellular proliferation [7]. Also, Yamamoto *et al.* [24] reported that cell proliferation rate of MCF-7 breast cancer cells was drastically decreased when FXYD3a and 3b mRNAs were suppressed by the small interfering RNA. In agreement with previous studies, the FXYD3-knockdown cells derived from SCC-4 and HO-1-U-1 OSCC cell line grew significantly slower than control cells in the current study. These data indicate that the FXYD could play an important role in the proliferation of oral cancer cells and FXYD3 downregulation might be an effective anti-cancer strategy in oral cancer.

In order to support our results on anti-proliferative effects of FXYD3 downregulation *in vitro*, we investigated the correlation between FXYD3 expression and proliferation in OSCC tissue samples. In the present study, FXYD3-strong group had significantly higher Ki-67 LI than FXYD3-weak group. These findings are consistent with our *in vitro* results, which the FXYD3-knockdown cells grew significantly slower than the control cells. Additional work is under way to determine signaling pathways and molecules responsible for the effect of FXYD3 downregulation on cell proliferation in OSCC cells.

In summary, we obtained two FXYD3-knockdown clones (SCC-4si and HO-1-U-1si) from the SCC-4 and HO-1-U-1 OSCC cell lines, in order to investigate the effect of FXYD3 downregulation on cell proliferation and migration. The FXYD3-knockdown cells grew significantly slower than the control cells. SCC-4si cells proliferated at 35.7%, 60.9% and 62.7% of the rate of control cells at 24, 48, and 72 hours after transfection, respectively. Also, HO-1-U-1si cells did at 47.7%, 28.3% and 34.5%, respectively. FXYD3-knockdown cells showed reduced migratory ability. The migration percentages of SCC-4si cells were 37.2% and 38.4% after 24 and 48h, respectively, compared with the control cells. Also, those of HO-1-U-1si cells were 33.3% and 44.1%, respectively. OSCC showed significantly higher FXYD3 expression than normal mucosa. The group with strong expression of FXYD3 had significantly higher Ki-67 labelling index than that with weak expression in OSCC tissue samples. These data suggest that the downregulation of FXYD3 induces anti-proliferative and anti-invasive effects in OSCC and that FXYD3 might be a useful target molecule for the treatment of OSCC.

V. Conclusion

These results suggest that the downregulation of FXYD3 induces anti-proliferative and anti-migratory effects in OSCC and that FXYD3 might be a useful target molecule for the treatment of OSCC.

VI. Reference

1. Sankaranarayanan R, Masuyer E, Swaminathan R. Head and neck cancer: a global perspective on epidemiology and survival. Anti Cancer Res 1998.18(6B):4779–4786.
2. Carvalho AL, Ikeda MK, Magrin J, Kowalski LP. Trends of oral and oropharyngeal cancer survival over five decades in 3267 patients treated in a single institution. Oral Oncol 2004;40(1):71–76.
3. Sites of recurrence in oral and oropharyngeal cancers according to the treatment approach. Oral Dis 2003;9(3):112–118.
4. Bettendorf O, Piffkò J, Bànkfalvi A. Prognostic and predictive factors in oral squamous cell cancer : important tools for planning individual therapy? Oral Oncol. 2004;40:110-119
5. Seeadner KJ, Rael E. The FXYD gene family of small ion transport regulators or channels : cDNA sequence, protein signature sequence, and expression. Genomics. 2000;68:41-56.
6. Morrison BW, Moorman JR, Kowdley GC, Kobayashi YM, Jones LR, Leder P. Mat-8, a novel phopholemman-like protein expressed in human breast tumors, induces a chloride conductance in Xenopus oocytes. J Biol Chem 1995;270:2176-2182.
7. Grzmil M, Voigt S, Thelen P, Hemmerlein B, Helmke K, Burfeind P. Up-regulated expression of the MAT-8 gene in prostate cancer and its siRNA-

mediated inhibition of expression induces a decrease in proliferation of human prostate carcinoma cell. Int J Oncol 2004;29:97-105.

8. Morrison BW, Leder P: Neu and ras initiate murine mammary tumors that share genetic markers generally absent in c-myc and int-2-initiated tumors. Oncogene 1994, 9:3417–3426.
9. Kayed H, Kleeff J, Kolb A, Ketterer K, Keleg S, Felix K, Giese T, Penzel R, Zentgraf H, Buchler MW, Korc M, Friess H. FXYD3 is overexpressed in pancreatic ductal adenocarcinoma and influences pancreatic cancer cell growth. Int J Cancer. 2006;118:43-54
10. Okudela K, Yazawa T, Ishii J, Woo T, Mitsui H, Bunai T, Sakaeda M, Shimoyamada H, Sato H, Tajiri M, Ogawa N, Masuda M, Sugimura H, Kitamura H. Down-regulation of FXYD3 expression in human lung cancers : its mechanism and potential role in carcinogenesis. Am J Pathol. 2009;175:2646-2656
11. Hong SD, Hong SP, Lee JI, Lim CY. Expression of matrix metalloproteinase-2 and -9 in oral squamous cell carcinomas with regard to the metastatic potential. Oral Oncol 2000;36(2):207-13.
12. Lim J, Kim JH, Paeng JY, Kim MJ, Hong SD, Lee JI, Hong SP. Prognostic value of activated Akt expression in oral squamous cell carcinoma. J Clin Pathol 2005;58(11):1199-205.
13. Zhu ZL, Zhao ZR, Zhang Y, Yang YH, Wang ZM, Cui DS, Wang MW, Kleeff J, Kayed H, Yan BY, Sun XF. Expression and significance of FXYD-3 protein in gastric adenocarcinoma. Dis Markers 2010;28(2):63-9.

14. Rajasekaran SA, palmer LG, Moon SY, Peralta Soler A, Apodaca GL, Harper JF, Zheng Y, Rajasekaran Ak. Na, K-ATPase activity is required for formation of tight junctions, desmosomes, and induction of polarity in epithelial cells. Mol Biol Cell 2001;12:3713-3732.

15. Palmer CJ, Scott BT, Jones LR. Purification and complete sequence determination of the major plasma membrane substrate for cAMP-dependent protein kinase and protein kinase C in myocardium. J Biol Chem 1991;266:11126-11130.

16. Forbush B 3rd, Kaplan JH, Hoffman JF. Characterization of a new photoaffinity derivativeof ouabain : labeling of the large polypeptide and of a proteolipid component of the Na, K-ATPase. Biochemistr. 1978;17:3667-3676.

17. Attali B, latter H, Rachamin N, Garty H. A corticosteroid-induced gene expressing an "IsK-like" K^+ channel activity in Xenopus oocytes. Proc Natl Acad Sci USA 1995;92:6092-6096.

18. Fu X, Kamps MP. E2a-Pbx1 induces aberrant expression of tissue-specific and developmentally regulated genes when expressed in NIH 3T3 fibroblasts. Mol Cell Biol 1997;17:1503-1512.

19. Yamaguchi F, Yamaguchi K, Tai Y, Sugimoto K, Tokuda M. Molecular cloning and characterization of a novel phopholemman-like protein from rat hippocampus. Brain Res Mol Brain Res 2001;86:189-192.

20. Béguin P, Crambert G, Monnet Tschudi F, Uldry M, Horisberger JD, Garty H, Geering K. FXYD7 is a brain-specific regulator of Na,K-ATPase alpha 1-beta isozymes. EMBO . 2002;21:3264-3273.
21. Zhang Z, Pang ST, Kasper KA, Luan C, Wondergem B, Lin F, Chuang CK, Teh BT, Yang XJ. FXYD3: A promising Biomarkers for Urothelial Carcinoma. Biomark Insights 2011;6:17-26.
22. Widegren E, Onnesjö S, Arbman G, kayed H, Zentgraf H, Kleeff J, Zhang H, Sun XF. Expression of FXYD3 protein in relation to biological and clinicopathological variables in colorectal cancers. Chemotherapy 2009;55:407-13.
23. Zhu ZL, Yan BY, Zhang Y, Yang YH, Wang MW, Zentgraf H, Zhang XH, Sun XF. Overexpression of FXYD-3 is involved in the tumorigenesis and development of esophageal squamous cell carcinoma. Dis Markers 2013;35(3):195-202.
24. Yamamoto H, Okumura K, Toshima S, Mukaisho K, Sugihara H, Hattori T, Kato M, Asano S. FXYD3 protein involved in tumor cell proliferation is overproduced in human breast cancer tissues. Biol Pharm Bull. 2009 Jul;32(7):1148-54.

Figure Legends

Fig. 1. Screening of OSCC cell line for FXYD3 expression by RT-PCR (upper and middle panals) and Western blot (lower panel). SCC-4 and HO-1-U-1 showed prominent FXYD3 expression.

Fig. 2. Confirmation of FXYD3 Knockdown by siRNA. RT-PCR (A) and Western blot (B) analysis showed that FXYD3 mRNA and protein were efficiently downregulated in SCC-4si and HO-1-U-1si.

Fig. 3. Anti-proliferative effects of FXYD3 downregulation. Cell proliferation assay showed that the FXYD3-knockdown cells grew significantly slower than the NSC-transfected control cells. SCC-4si cells proliferated at 64%, 39% and 37% of the rate of control cells at 24, 48, and 72 hours after transfection, respectively ($P<0.05$). Also, HO-1-U-1si cells did at 59%, 74% and 69%, respectively ($P<0.05$).

Fig. 4. Reduced migratory ability by FXYD3 downregulation. FXYD3-knockdown cells showed reduced migratory ability. The migration percentages of SCC-4si cells were 37.2% and 38.4% after 24 and 48h, respectively, compared with the NSC-transfected control cells ($P<0.05$). Also, those of HO-1-U-1si cells were 33.3% and 44.1%, respectively ($P<0.05$).

Fig. 5. Representative immunohistochemical analysis of FXYD3 and Ki-67 in OSCC, showing both (A) positive expression of FXYD3 and (B) high level of Ki-67 expression (original magnification x200).

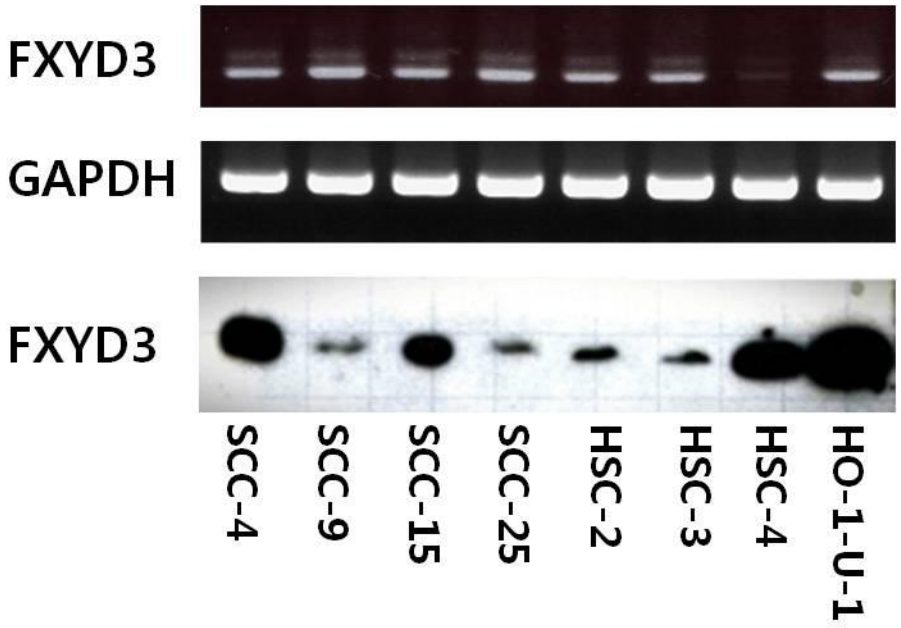
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Figure 1

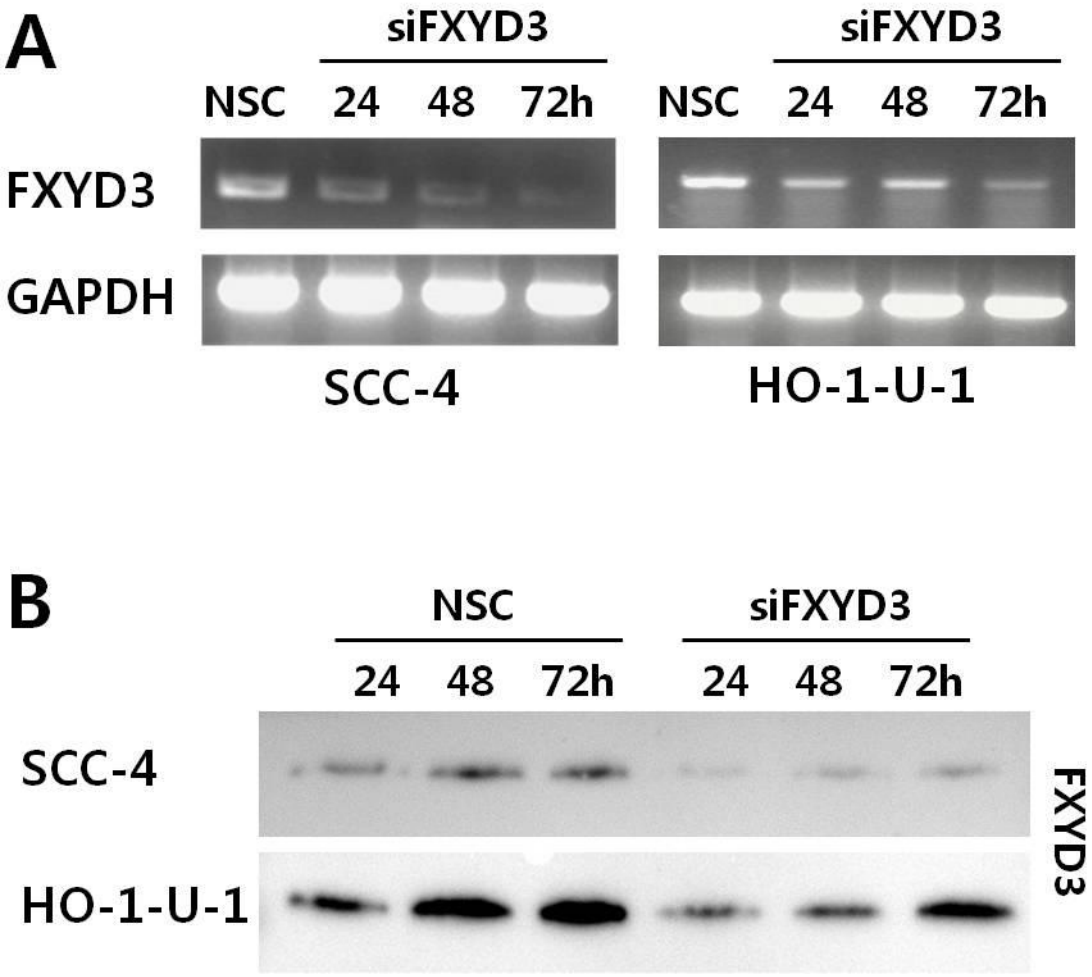
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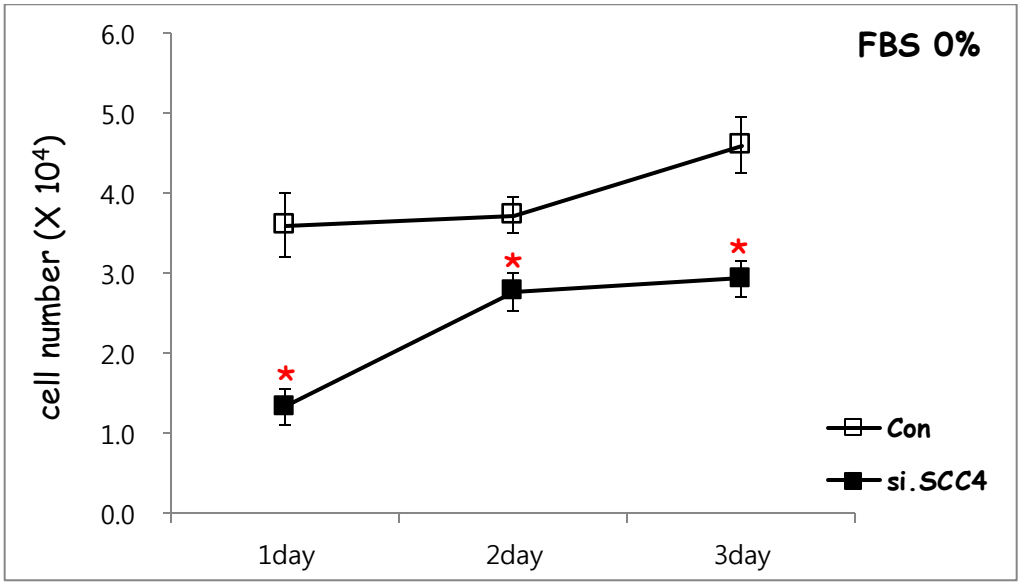
Figure 2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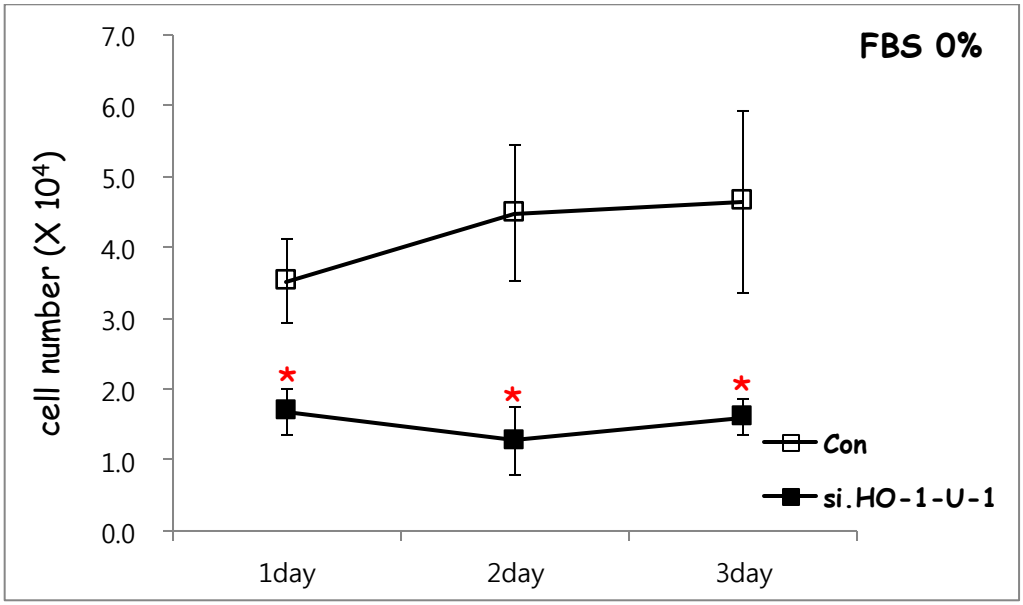
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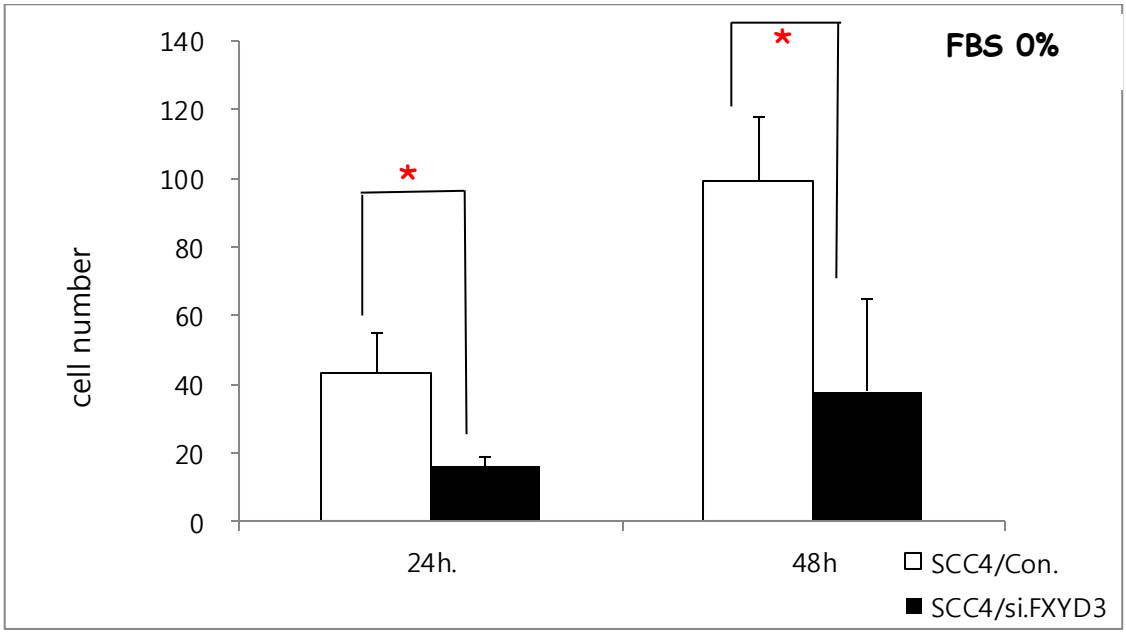
Figure 3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cell number
140
120
100
80
60
40
20
0
*
*
24h.
48h
SCC4/Con.
SCC4/si.FXYD3

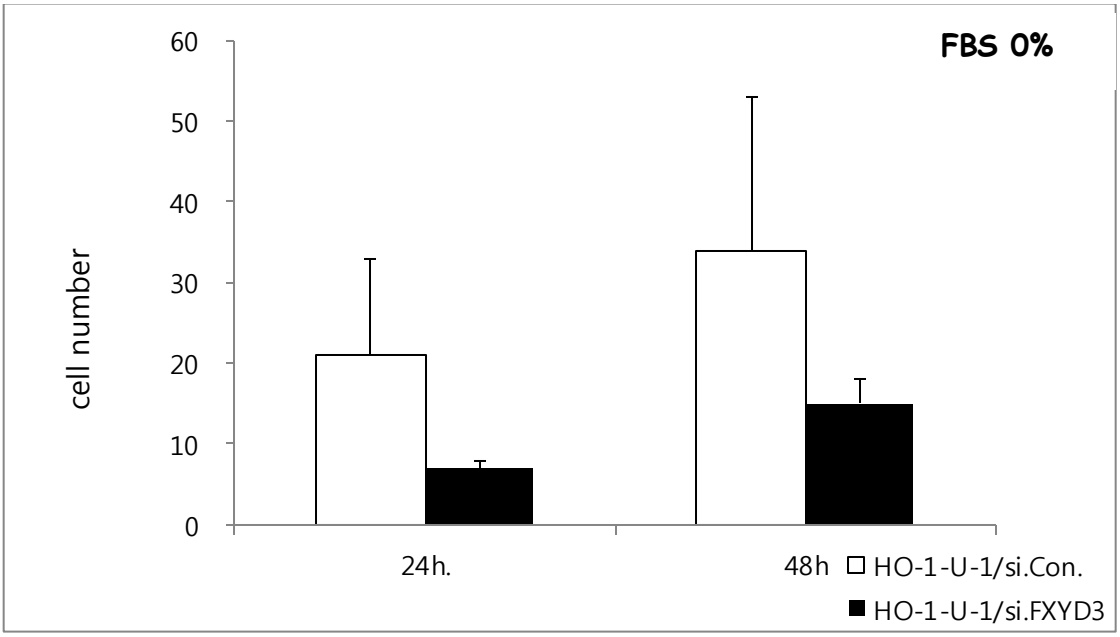
FBS 0%
cell number
60
50
40
30
20
10
0
24h.
48h
HO-1-U-1/si.Con.
HO-1-U-1/si.FXYD3

Figure 4

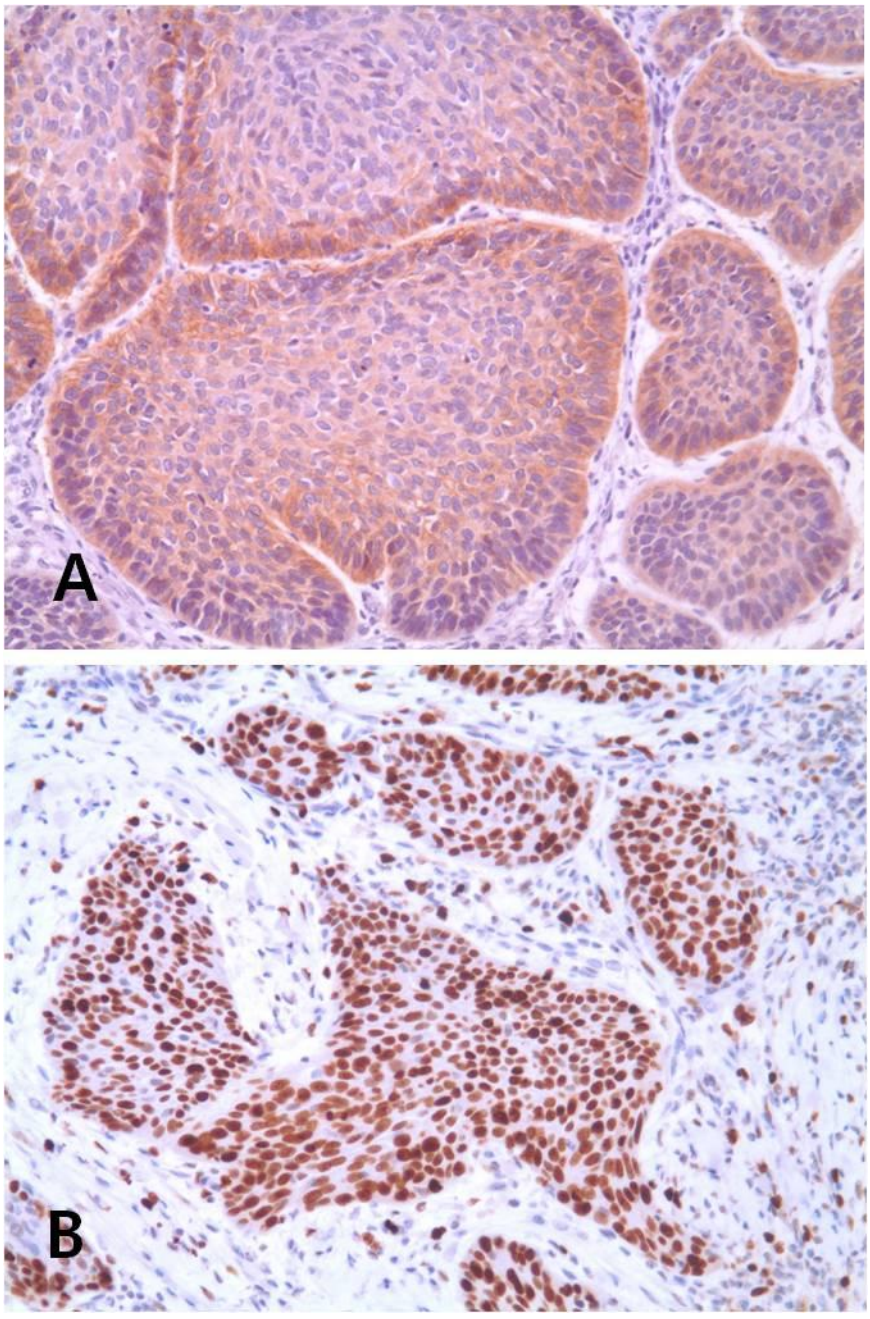

Figure 5

국문초록

FXYD3 발현 저하 유도가 구강편평세포암종에서 세포 증식과 이동 능력 억제 효과에 관한 연구

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구강 편평세포암종은 두경부암 중에서 가장 흔한 형태의 암종으로, 인류의 건강에 커다란 위협이 되고 있다. 최근 연구에 따르면, FXYD3 는 유방암, 전립선암, 그리고 췌장암에서 과발현되며, 따라서 종양형성에서 성장을 촉진하는데 역할을 한다고 보고되고 있다. 반면에 폐암에서는 FXYD3 의 발현이 정상조직과 비교하였을 때, 감소되었다고 보고된 바 있다. 이러한 보고들의 불일치를 미루어 보았을때, FXYD3 는 발암유전자 또는 종양 억제 유전자 역할 둘 다 할 수 있다는 것을 짐작할 수 있다. 하지만, 구강 편평세포 암종의 종양형성 과정에서 FXYD3 가 어떠한 역할을 하는지는 현재 보고된 바가 없다. 따라서, 본 연구에서 구강 편평세포암종 세포에서 FXYD3 의 발현 정도를 확인하고, FXYD3 의 발현 저하가 종양 형성에 관여하는 세포 증식능과 이주능에 어떠한 역할을 하는지 알아보고자 하였다.

구강 편평세포암종 세포주 SCC-4 와 HO-1-U-1 에 FXYD3 siRNA 를 형질주입시켜 FXYD3 가 발현저하된 세포들을 (SCC-4si 와 HO-1-U-1si) 획득하였다. 이 세포들에서 FXYD3 발현저하가 세포 증식능과 이주능에 미치는 영향을 확인하고자 cell proliferation assay 와 in vitro migration assay 를 시행하였다. 또한, 60 명의 구강 편평세포암종 환자의 조직 시편에서 FXYD3 발현과 증식 정도를 평가하기 위하여 면역조직화학적 분석을 하였다.

FXYD3 가 발현저하된 세포들은 대조군에 비해 증식능이 감소하였다. SCC-4si 세포들의 형질주입 24, 48, 72 시간 후의 세포 증식은 각각 대조군의 35.7%, 60.9%, 62.7%의 비율을 보였다 ($P<0.05$). HO-1-U-1si 들의 경우는 각각 47.7%, 28.3%, 34.5%를 나타냈다 ($P<0.05$). 또한, FXYD3 가 발현저하된 세포들은 감소된 이주능을 보였다. SCC-4si 세포들의 형질주입 24, 48 시간 후의 세포 이동 비율은 각각 대조군의 37.2%, 38.4%였다 ($P<0.05$). HO-1-U-1si 들의 경우는 각각 33.3%, 44.1%를 나타냈다 ($P<0.05$). 구강 편평세포암종 환자의 조직 시편에서 FXYD3 발현이 인접 정상 점막 조직에 비해 유의성 있게 높았다 ($P<0.05$). 구강 편평세포암종에서 FXYD3 고발현 군의 Ki-67 지수가 저발현 군에 비해 유의성 있게 높았다 ($P<0.05$). 이러한 결과들로 보아, FXYD3 의 발현저하는 구강 편평세포암종 세포의 증식능과 이주능에 대한 억제 효과를 나타내며, 구강 편평세포암종 환자의 치료 전략에 FXYD3 가 유용한 목표 물질일 가능성을 시사한다.

주요어 : 구강편평세포암종, FXYD3, siRNA, 증식능, 이주능

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