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두경부편평세포암의 보조 항암제로서의 AZD8931 (sapitinib)의 가능성에 대한 연구

The possibility of AZD8931 (sapitinib) as a new adjuvant chemotherapy of head and neck squamous cell carcinoma (HNSCC)

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A thesis of the Doctor's degree

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The Graduate School, Seoul National University

Department of Otorhinolaryngology, College of Medicine

Heejin Kim

Abstract

The possibility of AZD8931 (sapitinib) as a new adjuvant chemotherapy of head and neck squamous cell carcinoma (HNSCC)

Heejin Kim
Otorhinolaryngology, College of Medicine
The Graduate School
Seoul National University

Introduction: Despite of advance of chemotherapy and radiation therapy, the survival rate of advanced head and neck squamous cell carcinoma (HNSCC) remains relatively low, and the treatment was often encountered many problems. Numerous kinds of target therapy for HNSCC have been developed, and among the human epidermal growth factor (HER, ErbB) family, ErbB2 and ErbB3 is the most studied molecules for target agent. A sapitinib (AZD8931), a small molecule for tyrosine kinase inhibitor (TKI) was used as a pan-ErbB blocker. In this study, we aimed to identify the overexpression of ErbB2 and ErbB3 in HNSCC cell lines. Then, we tried to reveal the utility of sapitinib as an adjuvant chemotherapy for HNSCC in the animal study.

Methods: Six human HNSCC cell lines (SNU1041, SNU1066, SNU1076, PCI01, PCI13, PCI50) were used, and expression of ErbB2, ErbB3, and molecules associated with downstream pathway (Akt, MEK, ERK) were evaluated by using western blotting. Changes after adding EGF and/or sapitinib was evaluated in HNSCC cell lines by cell proliferation assay and cell cycle assay. In the animal study, tumor xenograft was implanted at the lateral tongue of 15 mice, and tumor growth was evaluated. When the tumor size reached to 2-3 mm, the tumor was grossly resected. After resection of the tumor, mice were divided into sapitinib treatment group and control group. After 2 weeks of medication, mice were sacrificed when they met the criteria of sacrifice or after 21 days, and then the whole tongues were harvested for evaluation.

Results: In the HNSCC cell line, ErbB2 and ErbB3 overexpression was identified. It was inhibited by 50 nM of sapitinib in cell proliferation assay, but it did not induce the apoptosis in the cell cycle assay. Adding EGF did not show any differences in inhibition of proliferation or cell cycle study. Administration of sapitinib also showed

improvement of survival in an animal model as a postoperative adjuvant chemotherapy, without statistical significance.

Conclusions: Sapitinib showed inhibition of cell proliferation in vitro and in vivo study, therefore we can expect the role of sapitinib as an effective target chemotherapy in the recurrent/metastatic HNSCC. Further large size studies would expected to support these preliminary results.

keywords : Carcinoma, squamous cell of the head and neck • ErbB Receptors • Receptor, ErbB-2 • Receptor, ErbB-3 • sapitinib • Chemotherapy, Adjuvant • Cell Proliferation

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Introduction

Worldwide, head and neck cancer is one of the most common cancers with estimated 800,000 cases annually with high morbidity and mortality rates. (1) As well known, most of cancers originated head and neck area is squamous cell carcinoma. In Korea, the five-year survival rate for cancer of the lips, oral cavity and pharynx was reported 62.2% in 2012 (2) and for oral cavity squamous cell carcinoma (OSCC) in early and well-localized stage 80% and in advanced stage below 40%. (3)

Head and neck squamous cell carcinoma (HNSCC) treatment often encounters many problems. Due to its complicate anatomy, surgery for HNSCC often be unable to get adequate margins. In addition, organs related to vital functions, such as swallowing and breathing can be destroyed, and also causes asthetic problems. Chemotherapy and radiation therapy greatly contribute in HNSCC treatment but their use is limited because of severe toxicity. For this reason, the advance of target therapy is expected to promote new effective treatments. Human epidermal growth factor receptor (EGFR, HER, ErbB) is the most well-known therapeutic target for HNSCC because most HNSCCs arise from the upper aerodigestive tract epithelium. Overexpression of EGFR is detected in more than 90% of HNSCCs (4-6) and is correlated to lymph node metastasis, recurrence, and poor overall survival. (4,7-10) There have been many studies on the ErbB family evaluating its role as a possible target for HNSCC therapy.

Among the ErbB family, ErbB2 is the most studied molecule in various cancers. Due to formation of ErbB2-ErbB3 complex, the interest about ErbB3 has been increased in various cancers including HNSCC. We did the inmmunohistochemistry staining for ErbB3 to the OSCC patients who showed the opposite prognosis in the previous unpublished study. We can also see the overexpression of ErbB3 in recurred OSCC patients comparing to non-recurred OSCC patients who were in same clinical staging and underwent same radical treatment (Figure 1). There have been developed many kinds of anti-ErbB family blocker such as tyrosine kinase inhibitor (TKI) or monoclonal antibody (mAb) for ErbB family. Sapitinib (AZD8931) is a one of pan-ErbB family blocker, which is a small TKI molecule reversible, equipotent inhibitor of EGFR, ErbB2, and ErbB3.

In our study, we aimed to reveal the overexpression of ErbB2 and ErbB3 in HNSCC cell lines. Then, we tried to reveal the utility of sapitinib as an adjuvant chemotherapy for HNSCC in the animal study.

Materials & Methods

Cell lines

As human HNSCC cell lines, SNU1041 (hypopharyngeal cancer), SNU1066 (glottis cancer), SNU1076 (subglottic cancer), PCI01 (recurrent laryngeal cancer), PCI13 (retromolar trigone cancer), and PCI50 (tongue cancer) cell lines were used. We have already shown from our experimental data that the SNU1041, SNU1076, and PCI01 cell lines had tumorigenic activity. (11) The SNU1041, SNU1066, SUNU1076 cell lines were established in our institute and provided to us. (12,13) The PCI01, PCI13, and PCI50 cell lines were obtained from the University of Pittsburgh. (14,15) These HNSCC cell lines were maintained in advanced RPMI1640 (Gibco; Grand Island, NY) medium supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, and penicillin/streptomycin in a 37ºC incubator with 5% CO_2.

Western blotting

The following antibodies were used for immunoblotting of cell lines: ErbB2 (Abcam, Cambridge, UK), phosphorylated ErbB2 (p-ErbB2; Santa Cruz Biotechnology, Dallas, TX, USA), ErbB3 (Abcam), phosphorylated ErbB3 (p-ErbB3; Abcam), Akt (Cell Signaling Technology, Danvers, MA, USA), phosphorylated Akt (p-Akt; Cell Signaling Techonology), MAPK/ERK kinase (MEK; Cell Signaling Technology), phosphorylated MEK (p-MEK; Cell Signaling Techonology), extracellular signal-regulated kinase (ERK; Santa Cruz Biotechonology), phosphorylated ERK (p-ERK; Cell Signaling Technology). Cells from our 6 HNSCC cell lines were plated at 5×10^6 cells per well in six-well plates. After growth, cells were washed with cold PBS and lysed in culture dishes using Pro-prep protein extraction solution (Intron Biotechnology, Seongnam, Korea). Total protein concentration was quantified, and twenty micrograms of protein were loaded on 4 %-15 % gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis gels. Blots were probed with antibodies.

Cell Proliferation Assay

Among the all HNSCC cell lines, we selected candidate cell lines which well-expressed ErbB2, ErbB3 and proteins associated with its downstream pathway in Western blot. Then, we excluded the cell lines which were known to have nontumorigenic activity in previous study. (11) Therefore, SNU1041 cell line and SNU1076 cell line would be candidate for our experiment. Finally, we selected the SNU1041 cell line for our *in*

vitro and *in vivo* experiments which derived from hypopharyngeal cancer. SNU1041 cells were plated in triplicate at a concentration of 2×10^3 cells per well into 96-well plates and incubated for 3 days. Then, sapitinib was applied at concentrations of 10, 20, 30, 40, and 50 nM, and control wells were also included. Detection was performed by the MTT assay after 24, 48, 72, and 96 hour incubation to identify the effect of sapitinib on SNU1041 cell line proliferation. The IC_{50} value, *i.e.* the concentration at which 50% of the cell growth is inhibited compared to the control, was calculated by nonlinear regression analysis using the GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA). Drug concentration for further analysis was determined by IC_{50} values.

Cell cycle analysis

Cell cycle analysis was performed by flow cytometry. Our HNSCC cells from SNU1041 cell line were plated in six-well dishes. Then, they were washed with PBS and detached with trypsin at 37°C. After another wash in PBS, the cells were divided in four groups and in each group 4 nM EGF, 50 nM sapitinib, 4 nM EGF with 50 nM sapitinib, and control were added respectively. Cells were fixed with 70% ethanol and stained with propidium iodide (PI) in the presence of RNase A. After incubation for 30 min, cell cycle analysis was performed using flow cytometry by the FACS Calibur and Cell Quest software (BD Biosciences, San Jose, California, USA).

***In vivo* animal model for post-surgery recurrence**

Animal studies were performed in accordance with the protocol approved by our *Instituitional Animal Care and Use Committee.* In our previous study, we made a tongue cancer animal model by tumor xenograft. (11) As our previous tongue cancer model, 1×10^6 cells from SNU1041 cell line in 15 μL of PBS were injected to the lateral tongues of 6-8 week-old nude mice. When the tumour size reached 2-3 mm, a gross mass of the tongue was removed by capsular dissection. Then, the experimental group was divided into two subgroups; the control group ($n=7$) and the sapitinib treatment group, which was a post-operative adjuvant therapy group ($n=8$). In the sapitinib treatment group, sapitinib was administered perorally for 2 weeks postoperatively; saline was administered instead to the control group during the same period. Drug was administered 5 days per week for 2 weeks by oral gavage in a concentration of 25 mg/kg. If the body weight of mice decreased more than 30 % of initial body weight (which measured when cancer cells injected to the lateral tongue),

the mice were sacrificed and harvested the whole tongue for evaluation. After 21 consecutive days, all the survived mice were sacrificed and their tongues were harvested for the evaluation of the local tumour recurrence (Figure 2). Among 15 mice, 3 mice died immediately after surgery. Therefore, 12 mice (6 in the control group and 6 in the sapitinib treatment group) were enrolled in this study.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 20.0 (SPSS Inc.; Chicago, IL) and Graph-Pad Prism 5 software programs. For the invaded cell number comparison the independent sample t test was used for the mouse analysis. The level of statistical significance was set at $p<0.05$.

Results

ErbB2 and ErbB3 expression in HNSCC cell lines

We tried to estimate ErbB2 and ErbB3 expression in HNSCC cell line by western blotting. In previous experiments and literature review, (13) SNU1066 and PCI13 were not suited as xenograft models in nude mice and had low tumorigenic potential. Total and p-ErbB2 expression was strong in the tumorigenic cell lines PCI01, PCI50, SNU1041, SNU1076, and SNU1066; ErbB3 was also strongly expressed in these cell lines. No expression was found in the non-tumorigenic cell line PCI13. However, p-ErbB3 expression in the SNU1066 cell line was low compared to the expression in SNU1041 or SNU1076 cell lines. p-Erk and p-MEK were also strongly expressed in these tumorigenic cell lines (Figure 3). As in a previous study, (16) ErbB2 overexpression could be associated with the expression of Erk and MEK by activation of the downstream pathways, in these cell lines (Figure 3).

Sapitinib inhibits the growth of SNU1041 cells

After 72 hours incubation in MTT assay, the 40 nM and 50 nM sapitinib groups showed a significant cell growth inhibition compared to the control group with IC_{50} level of 27.23 nM (Figure 4A and 4B). Subsequently, we used in our experiment the concentration of 50 nM which is twice as the IC_{50} level.

To identify sapitinib effect on ErbB2, ErbB3, EGFR, and other downstream effector molecules, 50 nM of sapitinib and/or 4 nM of EGF were added to SNU1041 cell line for 72 hour incubation. The addition of EGF did not affect the cell line behavior. The addition of sapitinib either with or without EGF significantly decreased the expression level of p-Akt. Whereas total MEK expression level was decreased in sapitinib group, p-MEK expression level did not show any difference by the sapitinib addition. Therefore, p-Akt pathway could play an important role in the decrease of cell proliferation by sapitinib (Figure 5).

In cell cycle analysis, the addition of sapitinib or EGF did not induce any changes of the percentage of the G0/G1-, S-, and G2/M phase cells in the HNSCC cells (Figure 6). Figure 7 presents the results of the MTT assay after administration of 50 nM sapitinib following 24-, 48-, 72-, and 96-hour incubation. When sapitinib was administered, the MTT absorbance level was significantly decreased after 72 hours.

Effect of sapitinib as a post-operative adjuvant chemotherapy *in vivo*

HNSCC cells from SNU1041 cell line were injected to the lateral tongue of mice, and tumor showed well-growth in all mice. After excision of the tongue tumor, acute weight loss was observed in both groups (control and sapitinib treatment group) (Figure 8). However, the mice in the sapitinib treatment group maintained their weight for 2 days after surgery. The 9th day after surgery, 4 mice from the control group and 2 mice from the sapitinib treatment group showed a large recurred tumor in the tongue and weight loss; then the mice were sacrificed. The remaining mice were followed-up for their weight and were sacrificed at the end of the experiment; the whole tongue was removed and sent for pathological examination. The survival curve showed some advantage of the sapitinib treatment group, but no statistical significance was observed (Figure 9). Because the mice were sacrificed by the criterion of the body weight loss, and the size of recurred tongue tumor was similar in both group of the end point (Figure 10). However, tumor of the control group showed faster tumor growth rate compared to those of the sapitinib treatment group (Figure 11).

Discussion

A representative target agent of ErbB family in head and neck cancer is cetuximab, a mAb that specifically blocks EGFR, and is the only one approved by the US Food and Drug Administration (FDA) for the treatment of HNSCC. Through EXTREME trial, cetuximab combined with platinum/5-fluorouracil has been considered as the standard treatment for recurrent or metastatic HNSCC (R/M HNSCC). (17-19) However, cetuximab has also limitations such as low efficacy and short duration remissions. (19) To overcome cetuximab resistance, numeral efforts have been made for the understanding of the underlined molecular mechanisms and the search for new target agents as a second-line treatment for R/M HNSCC. The understanding of ErbB signaling is included in those efforts. In the ErbB family, ErbB2 as a candidate for new target agents is the most studied molecule. Overexpression of EGFR and ErbB2 has been reported in various cancers, such as breast, colon, pancreas, and head and neck cancer. Overexpression of ErbB2 can cause proliferation signaling via PI3K/Akt or MAP kinase pathways, and enhance of cell growth and division. (20) It was reported that overexpression of ErbB2 in breast cancer confer radio-resistance to tumors, (21-24) generating the need for the use of an anti-ErbB2 antibody such as trastuzumab for clinical practice. In head and neck cancer, a phase II study revealed that treatment with lapatinib as an adjuvant therapy together with cisplatin-based chemotherapy in locally advanced, unresectable cancer showed a good response. (25)

ErbB3 has a non-functional kinase domain dissimilar to that of ErbB2. Therefore, ErbB3 plays a key function in ErbB2-mediated tumorigenesis by formation of an ErbB2-ErbB3 complex. (26) Compared to the other members of the ErbB family, there were fewer studies on ErbB3 owing to its moderate expression levels in cancer cells, and inability to form homodimer ErbB3-ErbB3 complexes. (26) In addition, except of the well-known role of ErbB3 at transactivation by dimerization, it also has unique features like ligand-independent endocytosis (27,29) and up-regulation when Akt pathway is inhibited. (29) In a meta-analysis, it was found that an association between the relatively high expression of ErbB3 and shorter survival of patients with breast, colorectal, pancreatic, melanoma, ovarian, and head and neck cancer was possible. (30) Furthermore, strong activation of ErbB2, ErbB3 and MET along with coupling to PI3K-Akt was seen in cetuximab resistant HNSCC cancer cells. (31) In our study, we also found expression of ErbB2 and ErbB3 in our HNSCC cell lines.

To overcome the limitation of only one ErbB family targeting monoclonal antibody, we selected a TKI which acting as a pan-ErbB family blocker. Due to its complex activation by heterodimerization, many kinds of TKI were investigated as dual/pan-erbB TKIs. Among the ErbB-directed TKIS, we selected a "sapitinib" which is oral equipotent inhibitor of EGFR, ErbB2, and ErbB3 signaling. In a previous study, sapitinib successfully inhibited EGFR, ErbB2, and ErbB3-mediated signaling in HNSCC *in vitro* and *in vivo* (32). In a study comparing other pan-ErbB TKIs (gefitinib and lapatinib) with sapitinib, the latter showed stronger tumor growth inhibition than the former. (32) Until now, most studies on sapitinib were focused to tumor inhibition efficacy in breast, colorectal and ovarian cancer, but there were lack of reports about head and neck cancer.

We added EGF to the HNSCC cell line for robusting the expression of ErbB family. A previous study demonstrated adding EGF can induced EGFR activation, and it also promotes activating interactions between EGFR and ErbB3 by their experimental data. (33) Addition of EGF to HNSCC cell line showed mild increase in expression of ErbB3, however there was no significant change of expression in other molecules including p-ErbB3. Adding EGF to sapitinib also did not show any significant inhibition.

Administration of sapitinib showed significant decrease in p-Akt expression at western blotting. Furthermore, MTT assay showed significant tumor inhibition by sapitinib. Then, we made a tongue cancer model of recurrent or remnant tumor after surgery, and tried to estimate sapitinib efficacy as an adjuvant therapy in HNSCC. After gross tumor excision without full margin, the mice treated with sapitinib tended to reduce tumor regrowth and maintained body weight compared to control group but without any statistical significance. Also, more mice survived until the endpoint in the sapitinib group compared to the control group. However, we did not get any statistical significant difference among the two groups as size of our animal study group was small. Considering the short survival of the control group, we assume that there current tongue cancer of the control group could grow faster than the cancer in the sapitinib treatment group.

Our study has several limitations including small study size, and bias for the accuracy of resection margin. We removed tumor xenograft by gross tumor with our naked eyes, so we did not confirm the clear resection margin. The bias of surgical margin would interfere with the assessment of the exact efficacy of tumor regrowth inhibition by sapitinib.

Despite these limitations, our study is valuable that we verified the role of sapitinib both in clinical settings and *in vitro/in vivo* experiments. We can deduce that blockage of pan-ErbB family would be useful for tumor growth inhibition in HNSCC. Furthermore, this is a unique study on sapitinib efficacy as an adjuvant chemotherapy in HNSCC. We made postoperative remnant HNSCC model *in vivo*, and treated with sapitinib as an adjuvant chemotherapy. Because most target agent used for R/M HNSCC as adjuvant therapy, these kinds of experiments can be worth to provide results as a preliminary data. From these promising results, we are encouraged to study further the anti-ErbB family agents as a new target chemotherapy for R/M HNSCC.

Conclusion

In our study, we can identify overexpression of ErbB2 and ErbB3 in HNSCC cell lines. Sapitinib showed inhibition of cell proliferation in vitro. In vivo study, we made a postoperative remnant HNSCC model, and administeration of sapitinib tend to inhibit tumor progression without statistical significance. From these results, we can expect the role of sapitinib as an effective target chemotherapy in the R/M HNSCC. To get the confidence of our study, further large size studies would expected to support these preliminary results.

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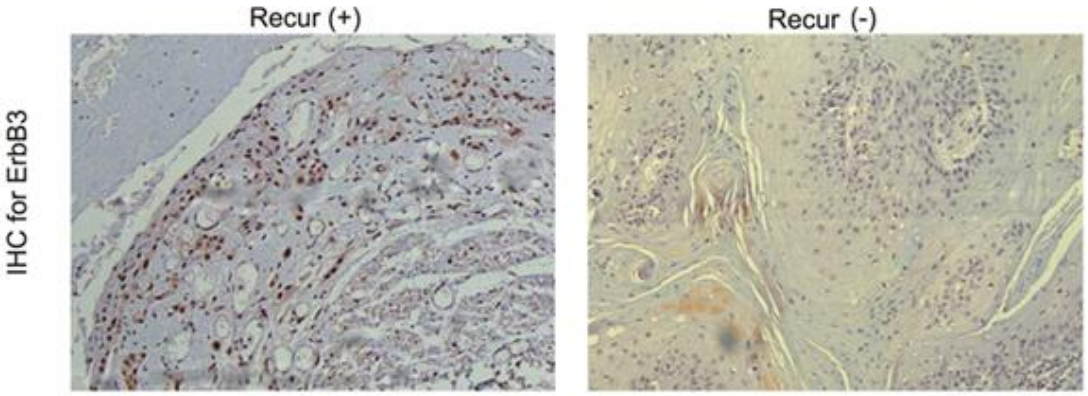


Figure 1. Previous results for immnunohistochemistry (IHC) staining of oral cavity squamous cell carcinoma (OSCC) patients. Patients were selected who showed same pathological staging (stage IV) at surgery and performed same radical treatment, but showed opposite prognosis. Recur group showed positive staining in the ErbB3 IHC staining.

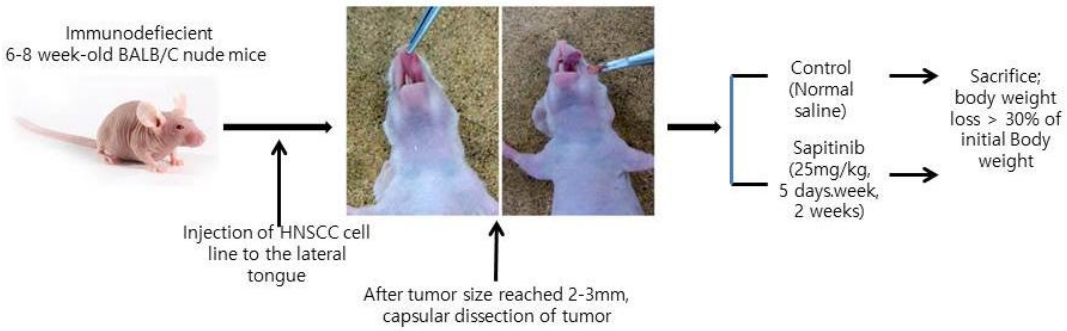


Figure 2. Experimental schedule in the mouse model. After injection of head and neck squamous cell carcinoma (HNSCC) cell line to the lateral tongue of the mice, tumor was grossly excised (capsular dissection) when its size reached to 2-3 mm. Then, we divided the groups into control (normal saline) and sapitinib treatment groups; sapitinib (25 mg/kg) or control vehicle was perorally for 5 times/week, 2 weeks. Mice were sacrificed when they exhibited any signs of morbidity or distress, experienced a weight loss > 30% of pre-injection body weight. After 21 consecutive days, all the survived mice were sacrificed and their tongues were harvested for the evaluation of the local tumour recurrence.

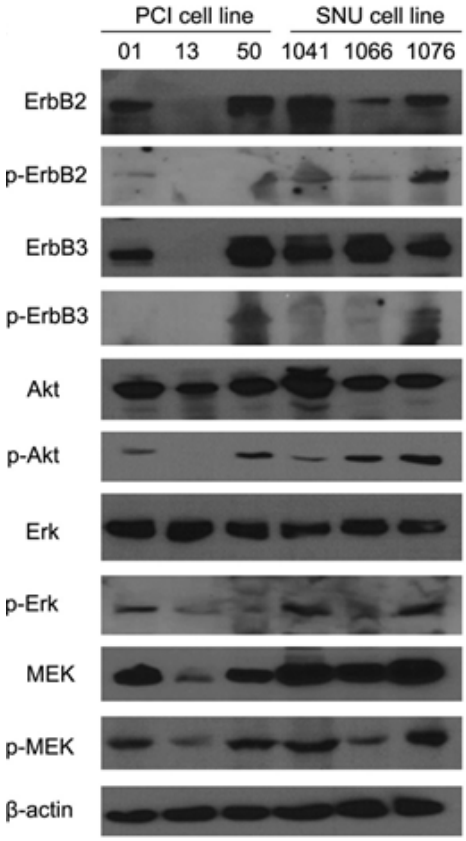


Figure 3. The protein expression in head and neck squamous carcinoma (HNSCC) cell lines (PCI01, PCI13, PCI50, SNU1041, SNU1066, SNU1076). Activated ErbB2 and ErbB3, and molecules associated with downstream pathways such as Erk and MEK was expressed in SNU1041, 1066, 1076 cell lines and PCI01, 50 cell lines.

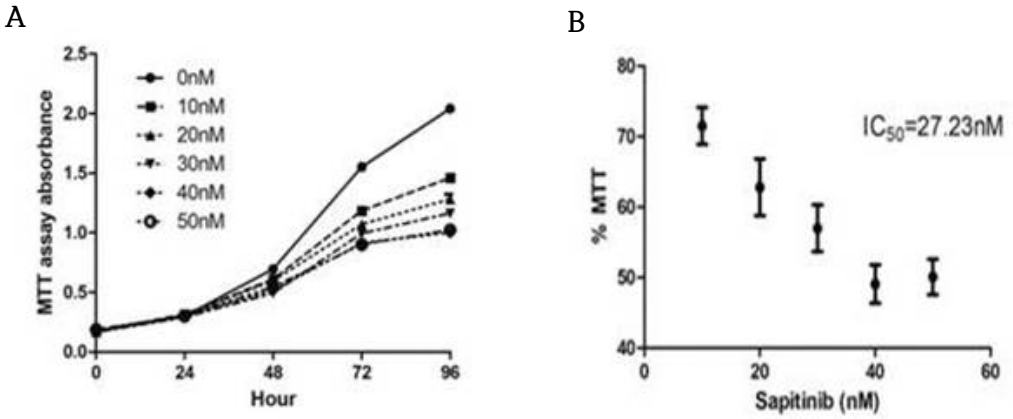


Figure 4. SNU 1041 cell line was treated with different concentration of sapitinib and detected using a MTT assay. After 72 hours, treatment groups of 40 nM and 50 nM sapitinib showed a significant cell growth inhibition compared to control group (A, B). Calculated IC_{50} was 27.23 nM (B).

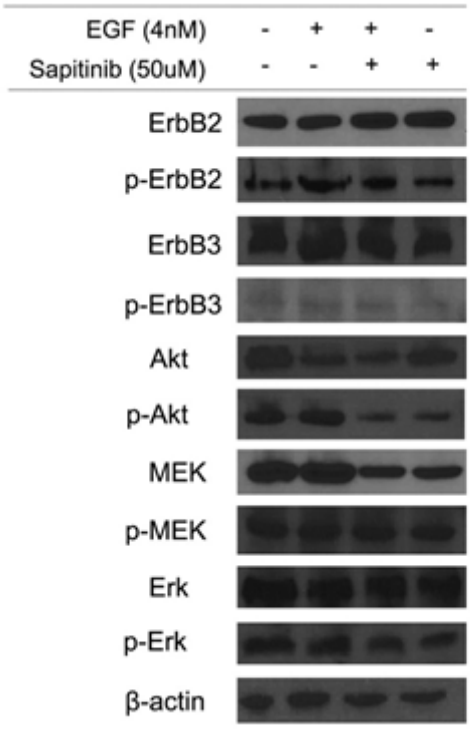
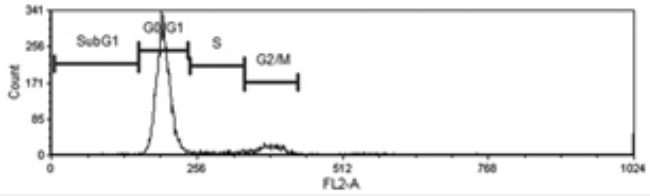


Figure 5. Changes of the protein expression in head and neck squamous carcinoma (HNSCC) cell lines after adding EGF and/or sapitinib. Adding 4 nM EGF in sapitinib gr C did not show any difference comparing to group treated with sapitinib only.



	subG1	G0/G1	S	G2/M
Control	1.22±0.16	75.60±2.08	5.47±0.26	12.63±1.92
EGF(4nM)	1.25±0.23	77.80±1.20	6.08±1.16	11.56±1.55
EGF(4nM)+sapitinib(50nM)	1.49±0.14	78.45±2.90	4.82±0.44	11.04±1.74
Sapitinib(50nM)	1.64±0.16	78.45±1.42	5.03±0.34	11.44±0.69
p-value (ANOVA)	0.031	0.237	0.111	0.557

Figure 6. Cell cycle analysis after adding EGF and/or sapitinib. In cell cycle analysis, adding sapitinib or EGF did not induce the apoptosis in our head and neck squamous cell carcinoma (HNSCC) cells.

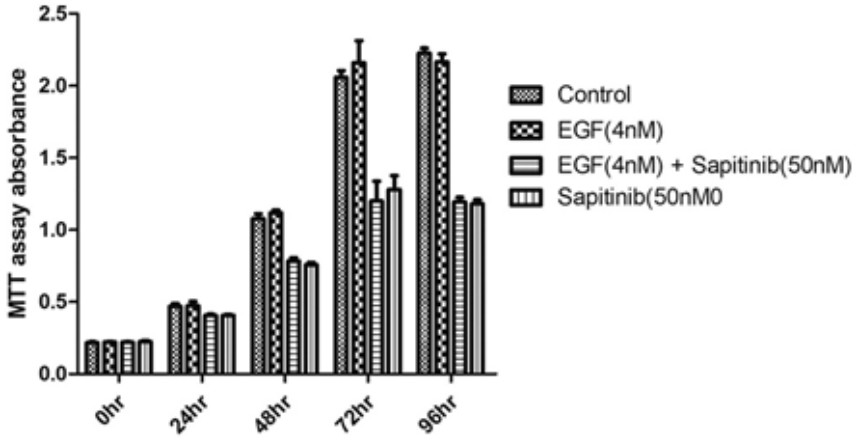


Figure 7. The result of MTT assay after adding EGF and/or sapitinib. When administering sapitinib, the MTT absorbances level was significant decreased after 72 hours.

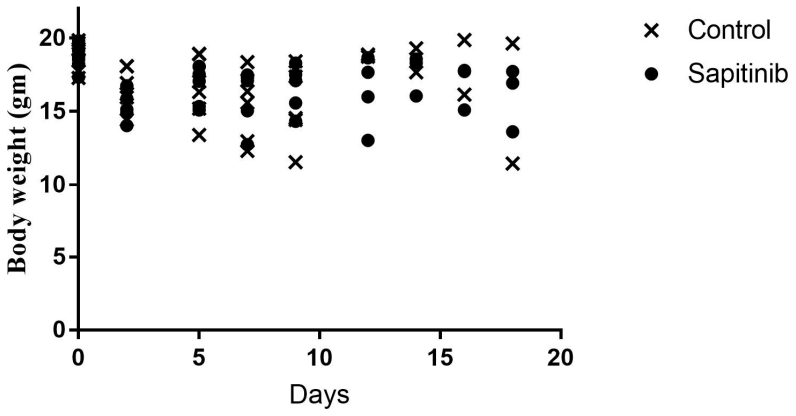


Figure 8. Changes of body weight of mice after grossly resection of the tongue tumor. After excision of the tongue tumor, acute weight loss was observed in both groups (control and sapitinib treatment group). However, the mice in the sapitinib treatment group maintained their weight for 2 days after surgery.

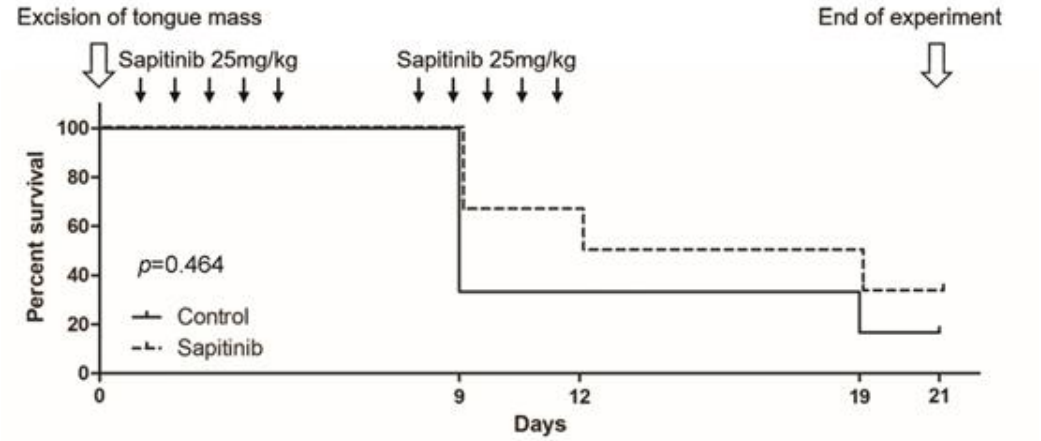


Figure 9. Effect of treatment with sapitinib as a post-operative adjuvant therapy in tongue cancer mice model. The survival curve showed some advantage of the sapitinib treatment group, but no statistical significance was observed.

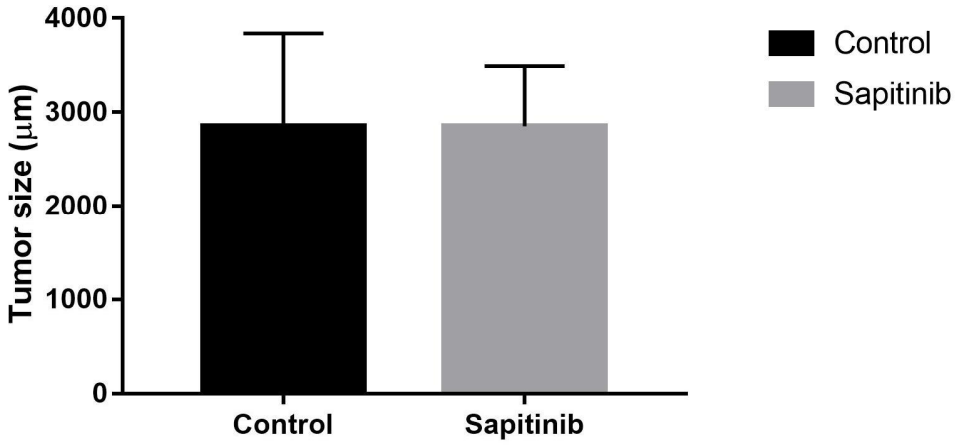


Figure 10. The tumor regrowth size after grossly resection of the tongue tumor. There was no statistical significant changes of tumor size between control group and sapitinib treatment group.

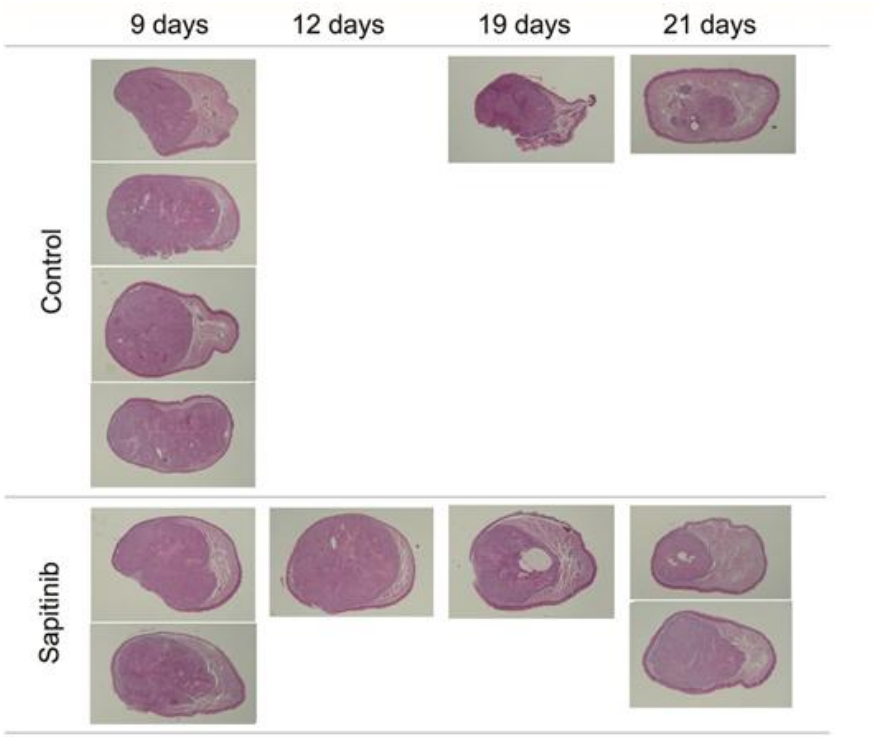


Figure 11. The time table of pathologic slides of regrowth tongue cancer after resection. After sacrificed the mice, whole tongue was resected and evaluated the regrowth of tongue cancer.

국문초록

서론: 항암치료와 방사선 치료의 발전에도 불구하고, 진행된 두경부암의 생존율은 상대적으로 낮으며, 치료는 종종 여러 문제에 부딪히게 된다. 다양한 종류의 두경부편평세포암의 표적 치료가 개발되어 왔으며, human epidermal growth factor (ErbB, HER) family 중 ErbB2와 ErbB3가 가장 많이 연구된 물질이다. Sapitinib은 작은 tyrosine kinase inhibitor으로 ErbB family 모두를 block하는 약제로 사용된다. 본 연구에서는 두경부 편평세포암의 세포주에서 ErbB2와 ErbB3의 발현을 알아보고, 이후 동물 실험을 통해 sapitinib의 adjuvant chemotherapy로의 유용성을 알아보고자 하였다.

방법: 총 6개의 두경부편평세포암 세포주 (SNU1041, SMU1066, SNU1076, PCI01, PCI13, PCI50)이 사용되었으며, ErbB2와 ErbB3, 그리고 downstream pathway와 관련된 물질들 (Akt, MEK, ERK)의 발현을 western blotting을 사용하여 평가하였다. 두경부편평세포암 세포주에서 EGF 혹은 sapitinib을 추가한 이후의 변화를 Cell proliferation assay와 cell cycle assay를 통하여 평가하였다. 이후 동물 실험에서, 종양 세포주를 15 마리의 쥐의 혀에 이식하여, 종양의 성장을 평가하였다. 종양의 크기가 2-3 mm 정도가 되면, 종양을 capsular dissection을 시행하였다. 종양을 절제한 후에 쥐를 sapitinib 치료 그룹과 control 그룹으로 나누어 관찰하였다. 2주간 투약한 이후, 쥐들이 sacrifice될 기준에 맞거나, 혹은 21일째가 되었을 때 모두 sacrifice하였고 이후 전체 혀를 적출하였다.

결과: 두경부편평세포암 세포주에서, ErbB2와 ErbB3의 과발현이 확인되었다. cell proliferation assay에서, 50 nM의 sapitinib을 투여하였을 때 세포의 성장이 저해되었으나, cell cycle assay에서는 apoptosis를 일으키지는 않았다. EGF를 추가하는 것은 cell proliferation이나 cell cycle 연구에서 유의한 차이를 보이지 않았다. 동물 실험에서도 sapitinib을 투여하는 것은 술 후 adjuvant 항암치료로써 생존율을 높이는 결과를 보였지만 통계적인 유의성은 없었다.

결론: Sapitinib은 두경부편평세포암 세포주 및 암 동물 모델에서 세포의 증식을 억제시키는 효과를 보여, 재발 혹은 전이성 두경부편평세포암에서 효과적인 target 제재로 sapitinib의 역할을 기대해볼 수 있다. 추후 더 많은 수의 연구를 통해 이 결과를 뒷받침할 수 있기를 기대한다.

주요어 : 두경부의 편평세포암종, ErbB receptors, ErbB-2 receptor, ErbB-3 receptor, sapitinib, 보조항암화학요법, 세포 증식

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