의학석사 학위논문

방사선 저항성 인간유두종바이러스 관련 두경부암에서 싸이클린 의존성 키나아제 4/6 억제제의 치료적 역할

Therapeutic role of CDK4/6 inhibitor in radioresistant HPV (+) head and neck squamous cell carcinoma

울산대학교대학원

의 학 과

이 동 규

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이 논문을 의학석사 학위 논문으로 제출함

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Summary

Background and Purpose

The clinical significance of human papilloma virus (HPV) related head and neck squamous cell carcinoma (HNSCC) has increased with its increasing incidence. HPV (+) HNSCC presents a better response to radiation therapy than HPV negative (-) HNSCC, however, some radioresistant cases have been reported. Abemaciclib, a selective cyclin dependent kinase (CDK) 4/6 inhibitor, is one of the emerging target agents of HPV (-) HNSCC, but not recommended primarily for the treatment of HPV (+) HNSCC. We hypothesized that the expression of Rb could be increased in radiation-resistant HPV (+) HNSCC, and the therapeutic efficacy of abemaciclib would be altered. In this study, we attempted to evaluate the radiation-induced changes in the Rb pathway and the effect of abemaciclib on radioresistant HPV (+) HNSCC.

Methods

In this *in vitro* study, 6 cell lines with different HPV infection status and p53 mutation status were prepared (HN30, UMSCC74A, UDSCC2, UMSCC47, UMSCC38, and HN8). The isogenic radioresistant cancer cell lines were established by performing serial fractionated irradiation. In each cell lines, the expression levels of cell cycle related proteins, such as Rb, p-Rb, p53, p21, p16, and Cyclin D1, were evaluated by western blot. The antitumor effects

of abemaciclib monotherapy and combined treatment with conventional chemotherapy drugs were evaluated using Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan).

Result

In HPV (+) cell lines (UDSCC2, UMSCC47), radioresistant cell lines presented higher Rb, p-Rb, and cyclin D1 expression than primary cell lines. In HPV (-) cell lines (HN30, UMSCC74A, UMSCC38, HN8), there was no significant difference in the cell cycle-regulating proteins. With abemaciclib monotherapy, HPV (+) radioresistant cell lines show lower cell viability than primary cell lines. Evaluating the response of abemaciclib combined with conventional chemotherapy agent, cisplatin and docetaxel combination therapy was more effective in HPV (+) radioresistant cell lines than primary cell lines, respectively. The differences in Rb/p-Rb expression could have a close correlation with the abemaciclib effect.

Conclusion

Radioresistant HPV (+) cell lines had higher expression of Rb, p-Rb, and cyclin D1 than primary HPV (+) cell lines. Both abemaciclib monotherapy and combination therapy had better antitumor effect in radioresistant HPV (+) cell lines than primary HPV (+) cell lines. Abemaciclib may be another treatment option for radioresistant HPV (+) HNSCC.

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Introduction

The prognosis of some types of head and neck squamous cell carcinomas (HNSCC), such as oropharynx cancer, is affected by the integration status of human papilloma virus (HPV).[1,2] The incidence of oropharynx cancer has increased with the increment of HPV-related oropharynx cancer patients while that of tobacco-related cancer has decreased. In the 1980s, the proportion of HPV-related HNSCC was only 20%; however, in 2005, this proportion increased to 70%.[3] Thus, the clinical significance of HPV-related HNSCC has increased in recent years and related studies are being conducted in the head and neck cancer field. Compared to HPV(-) HNSCC, HPV (+) HNSCC presents a unique pattern. HPV (+) HNSCC tends to be identified at the advanced stage, with small primary tumor size (low T-stage) and high nodal stage.[4,5] From a radiological viewpoint, HPV (+) HNSCC usually has well-defined, cystic nodal involvement and could have a small primary tumor.[6] Treatment response to either surgery or concurrent chemoradiation therapy is better for HPV (+) HNSCC than HPV (-), which led to the modification of TNM staging and the classification of HPV (+) cancer as a distinct category of HNSCC at the 8th edition of American Joint Committee on Cancer.

In HPV positive (+) HNSCC, HPV types 16 and 18, which are high-risk virus types of carcinogenesis, are mainly identified.[7] HPV infection in the epithelium produced oncoproteins E6 and E7, which downregulate the function of p53 and Retinoblastoma (Rb), respectively. Rb is a tumor suppressor protein that has an inhibitory effect on the cell cycle

by regulating E2F and control the transition from G1 to the S phase. In the physiologic Rb pathway, p16 inhibits the cyclin D1/ cyclin dependent kinase (CDK) 4/6 complex, which phosphorylates Rb and induces the release of E2F. E2F enters the nucleus and promotes cell cycle. Phosphorylated Rb (p-Rb) inhibits P16 expression as negative feedback[8] (Figure 1A). In HPV (+) HNSCC, E7 induces ubiquitin degradation of Rb, and E2F is released without the action of cyclin D1.[9] E2F causes cell cycle dysregulation and carcinogenesis. As Rb is not phosphorylated but degraded, the p16 inhibitory signal of p-Rb is decreased. Therefore, p16 expression is increased in HPV (+) HNSCC and is used as a biomarker (Figure 1B). The prognosis of HPV (+) HNSCC is more dependent on p16 (+) status than HPV infection status, which is detected either by DNA chip or PCR-based test.[10]

HPV (+) HNSCC presents a better response to radiation therapy than HPV negative (-) HNSCC.[11] However, some radioresistant cases have been reported. According to the 2021 National Comprehensive Cancer Network guideline, the remaining treatment options for residual and recurrent disease are salvage operation or chemotherapy; however, the prognosis is poor.[12] For patients who underwent salvage operation, mean overall survival was 25 months.[13] Further, their response rate (a complete response or partial response) to cisplatin, 5-FU, and cetuximab combination therapy was only 36%.[14] Several new target agents are being developed for these intractable cases; however, to date, the therapeutic results have not been promising.

Abemaciclib, a selective CDK 4/6 inhibitor, is one of the recently developed target agents for HPV (-) HNSCC.[15] Abemaciclib regulates the cell cycle by inhibiting the Cyclin D1-CDK 4/6 complex from the phosphorylation of Rb and blocks the transition from the G1 to S phase of the cell cycle. In HPV (+) cancer, E7 degrades Rb regardless of the action of CDK 4/6. Thus, the CDK 4/6 inhibitor is not effective and is not recommended primarily for the treatment of HPV (+) HNSCC. HPV (-) tumors showed partial remission following clinical trials with the CDK 4/6 inhibitor; however, HPV (+) tumors showed no response or disease progression.[16,17] A study to determine the effect of abemaciclib is conducted with recurrent and metastatic HNSCC patients who are resistant to platinum-based treatment (NCT03356587), but there was no additional study of the clinical efficacy of abemaciclib for HPV (+) HNSCC.

Recently, it has been reported that the expression of Rb increase after radiotherapy in HPV (+) uterine cervical cancer.[18] We hypothesized that the same changes could occur in the Rb pathway in radiation-resistant HPV (+) HNSCC, and the therapeutic efficacy of abemaciclib would be altered. In this study, we attempted to evaluate the radiation-induced changes in the Rb pathway and the effect of abemaciclib on radioresistant HPV (+) HNSCC.

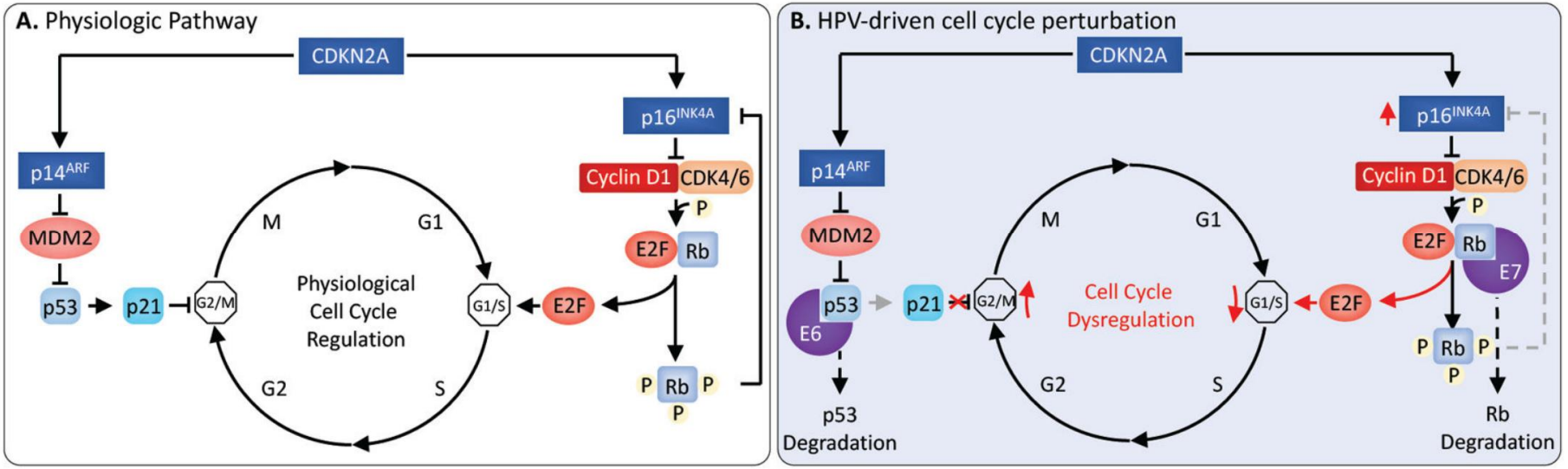

Figure 1. Carcinogenesis mechanism of human papilloma virus

A. In the physiologic Rb pathway, CDK2NA gene encodes p16 proteins. p16 inhibits the cyclin D1- CDK4/6 complex, which phosphorylates Rb and induces the release of E2F. E2F enters the nucleus and promotes the cell cycle. Phosphorylated Rb (p-Rb) inhibits P16 expression as negative feedback.

B. In HPV (+) HNSCC, E7 induces ubiquitin degradation of Rb, and E2F is released without the action of cyclin D1. E2F causes cell cycle dysregulation and carcinogenesis. The p16 inhibitory signal of p-Rb is decreased. p16 expression is increased in HPV (+) HNSCC

Methods

In this *in vitro* study, 6 cell lines with different HPV infection status and p53 mutation status were prepared (HN30, UMSCC74A, UDSCC2, UMSCC47, UMSCC38, and HN8). The HPV and p53 statuses of each cell line are shown in Table 1. HN30 (wild type p53; pharynx) cells were provided by Dr. Jeffrey N. Myers, University of Texas, MD Anderson Cancer Center, under a material transfer agreement. The UMSCC74A (wild type p53; tongue), UMSCC47 (HPV16 integrated, tongue), and UMSCC 38 (p53 mutation-R280K, oropharynx) cell lines were provided by Dr. Thomas N. Carey, University of Michigan. UDSCC2 (HPV 16 integrated, hypopharynx) cell lines were provided by Dr. Henning Bier, University of Dusseldorf. Finally, the HN8 (p53 mutation- G293 del; larynx) cell line was established at Asan Medical Center, Seoul, Korea. These cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin/streptomycin (Thermo Fisher Scientific), and incubated at 37 °C with 5% CO_2 in a humidified incubator. [19)]

The isogenic radioresistant cancer cell lines were established by performing serial fractionated irradiation. Serial fractionated irradiation for R-cells was performed as described below. Briefly, cells grown to 50% confluency were exposed to 2 Gy of X-ray radiation. When the cells reached 70−80% confluency, they were subcultured into a new dish and then irradiated with 2 Gy up to a cumulative dose of 70 Gy. The 6 primary

cell lines were called P-cells, and the isogenic radioresistant cell lines were called R-cells. [20]

In each P-cell and R-cell, the expression levels of cell cycle related proteins, such as Rb, p-Rb, p53, p21, p16, and Cyclin D1, were evaluated by western blot. We evaluated the difference in the signal pathway between P-and R-cells. Total protein was extracted using RIPA buffer supplemented with protease and phosphatase inhibitors (Thermo Fisher Scientific, Waltham, MA, USA). Protein concentrations were evaluated using a Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein were separated by SDS-PAGE and transferred to nitrocellulose membranes (GE healthcare, Freiburg, Germany). Membranes were incubated with primary antibodies against p53 (sc-126; SantaCruz, Dallas, TX, USA), p-RB (9308s), RB (9309S), p21 (2947S; Cell Signaling Technology, Danvers, MA), p16 (ab81278), cyclin D1 (ab134175; Abcam, Cambridge, MA, USA), and β-actin (A5441; Sigma-Aldrich, Inc., St. Louis, MO, USA). The secondary antibodies were goat anti-mouse or goat anti-rabbit IgG heavy and light chain antibodies (A120-101P and A90-116P; Bethyl Laboratories, Montgomery, TX, USA). Detection was performed with a Super Signal West Pico Trial kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Western blot analysis was performed at least three times, and representative figures are presented. [21]

We evaluated the response to abemaciclib in each P-cell and R-cell. The antitumor effects of abemaciclib monotherapy and combined treatment with conventional

chemotherapy drugs were evaluated using Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan). The cells (2000 cells/well) were incubated with cisplatin (MedChem Express, Monmouth Junction, NJ, USA), docetaxel (Sigma-Aldrich, St. Louis, MO, USA), and abemaciclib (MedChem Express, Monmouth Junction, NJ, USA) at 37 °C for 72 hours. Thereafter, 10 μl CCK-8 was added to each well. The plates were then incubated at 37°C for 1–4 hours. Absorbance was measured at 450 nm using an enzyme immunosorbent assay (ELISA) reader (Molecular Devices Co., Sunnyvale, CA, USA). [22)]

Statistical analyses were performed using Prism 7 software (GraphPad). One-way and two-way analysis of variance (ANOVA) tests were used to determine the statistical significance. A P-value of 0.05 or less was considered to indicate statistical significance. Statistically significant results are indicated in the figures.

Table 1. Characteristics of cell lines

Cell lines	P53-status	HPV-status
HN30	Wild type	negative
UMSCC74A	Wild type	negative
UDSCC2	Wild type	positive
UMSCC47	Wild type	positive
UMSCC38	R280K	negative
HN8	G293del	negative

Result

Rb is downregulated in HPV (+) P-cells and treatment efficacy of CDK4/6 inhibitor to HPV (+) P cells was diminished.

Western blot was performed to evaluate the expression of cell cycle regulatory proteins in HPV (+) and HPV (-) P-cells. The expression levels of Rb, p-Rb, and cyclin D1 were more downregulated in HPV (+) P-cells (UDSCC2, UMSCC47) than HPV (-) P-cells. Further, the expression of p16 was increased in HPV (+) P-cells (Figure 2A). The Rb/pRb/p-16 pathway, which was described in a previous study, was confirmed in the HPV (+) cell lines in our study.

We proceeded to explore the treatment efficacy of abemaciclib in the HPV (+) and HPV (-) cell lines. The response to abemaciclib monotherapy in P-cells is presented in Figure 2B. As expected, HPV (+) P-cells with downregulated Rb showed higher cell viability and IC_{50} than HPV (-) P-cells. The IC_{50} (μM) in each cell line was 0.27 (HN30), 0.32 (UMSCC74A), 1.74 (UDSCC2), 1.84 (UMSCC47), 0.33 (UMSCC38), and 0.79 (HN8). Thus, abemaciclib presented limited antitumor effect in HPV (+) P-cells compared to HPV (-) P-cells.

Establishment of isogenic radioresistant cancer cells (R-cells)

After establishing R-cells via serial fractionated irradiation, we evaluated the radiosensitivity using a clonogenic assay. The surviving fraction in 9 Gy radiation was

0.002 in HN30 P-cell and 0.01 in HN30 R-cell. Likewise, the other R-cells had a high surviving fraction than P-cells (Figure 3): 0.0007 and 0.0251 in UMSCC74A and R-cell; 0.0039 and 0.0228 in UDSCC2 and R-cell; 0.0002 and 0.0037 in UMSCC47 and R-cell; 0.0302 and 0.1487 in UMSCC38 and R-cell; 0.0037 and 0.0114 in HN8 and R-cell. Thus, we confirmed that the isogenic radioresistant cell was well established.

Expression of Rb, p-Rb, and cyclin D1 recovered in HPV (+) R-cells

The expression level of Rb, pRb, p53, p21, p16, and cyclin D1 between P-cells and R-cells was compared to evaluate the radiation-induced changes in the Rb-related pathway (Figure 4). In HPV (-) cell lines (HN30, UMSCC74A, UMSCC38, HN8), there was no significant difference in the cell cycle-regulating proteins between P-cells and R-cells. In HPV (+) cell lines (UDSCC2, UMSCC47), R-cells presented higher Rb, p-Rb, and cyclin D1 expression than P-cells. The expression of p53 and p21 also increased in HPV (+) R-cells. The expression of p16 decreased in UMSCC47 R-cells, but was stationary in UDSCC2 R-cells.

Antitumor effect of abemaciclib monotherapy in P-cells and R-cells

To compare the antitumor effects of abemaciclib in each cell line, the relative cell viability and IC_{50} were evaluated via the CCK-8 assay (Figure 5). No significant difference in HN30 and HN8 was found between P-cells and R-cells. The IC_{50} of abemaciclib in each

cell line was 0.281 (HN30), 0.283 (HN30-R), 0.784 (HN8), and 0.775 (HN8-R). Meanwhile, IC_{50} and cell viability were decreased in UMSCC74A-R, UDSCC2-R, and UMSCC47-R compared with each p-cell: IC_{50}: 0.331 (UMSCC74A), 0.163 (UMSCC74A-R), 1.725 (UDSCC2), 0.752 (UDSCC2-R), 1.828 (UMSCC47), and 1.0 (UMSCC47-R). In UMSCC38, R-cell displayed the worse response to abemaciclib relative to P-cell: IC_{50}: 0.329 (UMSCC38) and 0.464 (UMSCC38-R). These results suggests that abemaciclib had better antitumor effect in HPV (+) R-cells than P-cells.

Additive antitumor effect of abemaciclib combined with cisplatin and docetaxel in HPV (+) R-cells

In the clinical setting, cisplatin and docetaxel have been widely used to treat recurrent/persistent head and neck cancer.[12] Currently, several clinical trials are ongoing to evaluate the combination treatment of conventional chemotherapy with other types of chemotherapeutic agents.[16] In this study, we also assessed the additional antitumor effect of combination treatment with abemaciclib for radioresistant HPV (+) cells.

First, we evaluated the effect of abemaciclib combined with cisplatin and docetaxel by the CCK-8 assay. In UDSCC2 P-cell, low-dose (0.1 μM) abemaciclib combined with cisplatin had no additive effect; however, high-dose (1 μM) abemaciclib displayed an additive antitumor effect relative to cisplatin monotherapy. In UDSCC2 R-cell, not only high-dose abemaciclib, but also low-dose abemaciclib combination therapy displayed an

additional effect. High-dose abemaciclib combined with cisplatin showed a better additive effect than low-dose abemaciclib combination therapy in dose-dependent manner (Figure 6A). In both UMSCC47 P-cell and R-cell, abemaciclib combined with cisplatin showed an additive effect only at a high dose. High-dose abemaciclib had a better effect in UMSCC47 R-cell than P-cell. (Figure 6B). In summary, cisplatin and abemaciclib combination therapy was more effective in HPV (+) R-cells than P-cells.

We proceeded to evaluate the effect of abemaciclib combined with docetaxel. Abemaciclib combined with docetaxel did not result in an additive effect compared with docetaxel monotherapy in both UDCC2 and UMSCC47 P-cells. However, in UDSCC2 R-cell, both low-dose and high-dose abemaciclib combined with docetaxel showed a dose-dependent additive effect. In UDSCC47 R-cell, abemaciclib had an additive effect only at a high dose. In summary, docetaxel and abemaciclib combination therapy was more effective in HPV (+) R-cells than P-cells (Figure 7A, 7B).

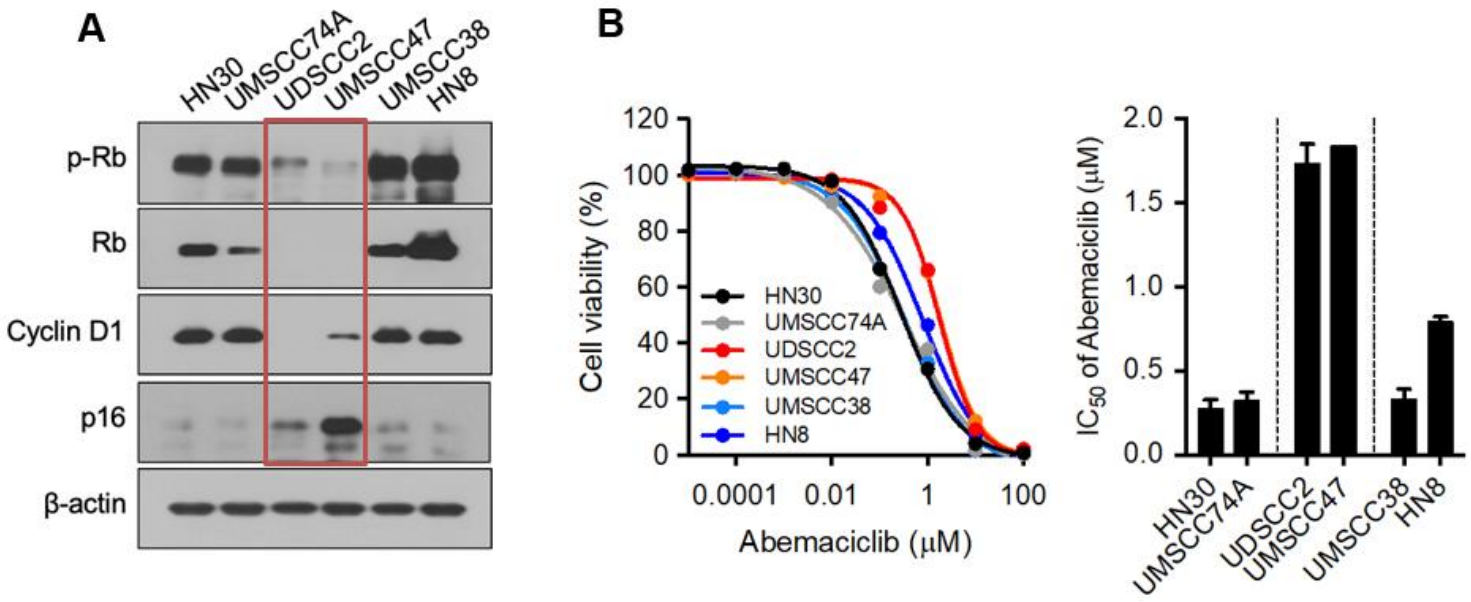

Figure 2. Expression of cell cycle related proteins and the antitumor effect of abemaciclib in P-cells

A. In HPV (+) P-cells (UDSCC2, UMSCC47), Rb, p-Rb, and cyclin D1 expression was downregulated, and p16 was overexpressed relative to levels in HPV (-) P-cells (HN30, UMSCC74A, HMSCC38, HN8)

B. HPV (+) P-cells with downregulated Rb showed higher cell viability and IC_{50} than HPV (-) P-cells. The IC_{50} (μM) of abemaciclib in each cell line was 0.27 (HN30), 0.32 (UMSCC74A), 1.74 (UDSCC2), 1.84 (UMSCC47), 0.33 (UMSCC38), and 0.79 (HN8).

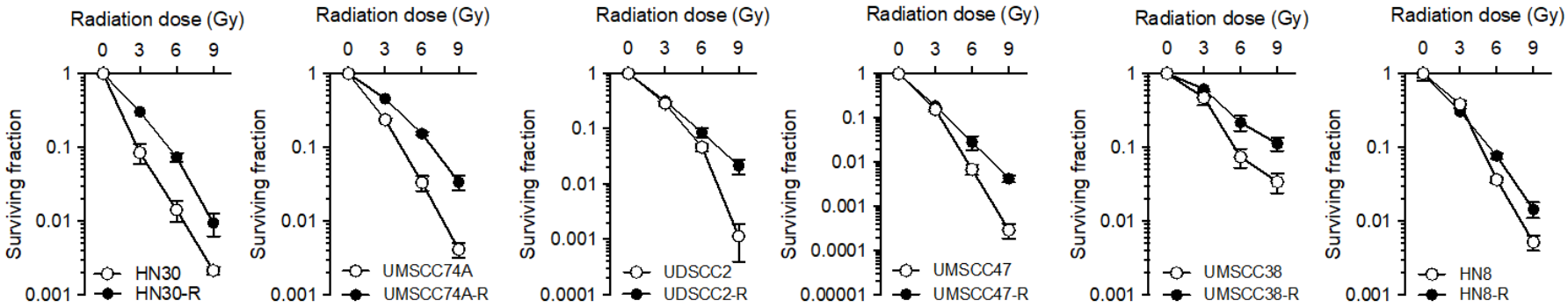

Figure 3. Surviving fractions of P-cells and the isogenic radioresistant cell line

The isogenic radioresistant cancer cell lines were established by performing serial fractionated irradiation. After establishing R-cells, the surviving fraction based on the accumulated radiation dose was evaluated in P-cells and R-cells using the CCK-8 assay.

The surviving fraction in 9 Gy radiation: 0.002 and 0.01 in HN30 and R-cell; 0.0007 and 0.0251 in UMSCC74A and R-cell; 0.0039 and 0.0228 in UDSCC2 and R-cell; 0.0002 and 0.0037 in UMSCC47 and R-cell; 0.0302 and 0.1487 in UMSCC38 and R-cell; and 0.0037 and 0.0114, in HN8 and R-cell.

All R-cells showed high surviving fraction relative to P-cells at the same radiation dose. Thus, we confirmed that the isogenic radioresistant cell was well established.

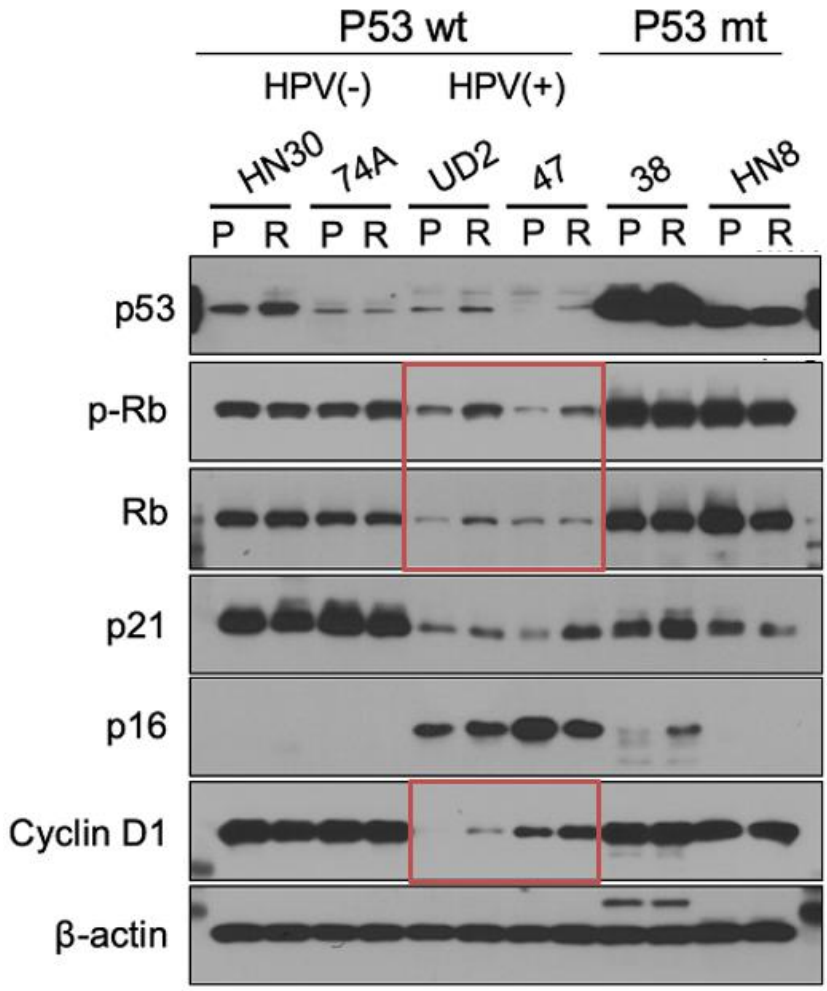

Figure 4. Expression of the cell cycle related proteins of P-cells and R-cells

In HPV (-) cell lines (HN30, UMSCC74A, UMSCC38, HN8), there was no significant difference in the cell cycle-regulators between P-cells and R-cells. In the HPV (+) cell lines (UDSCC2, UMSCC47), R-cells presented higher Rb, p-Rb, cyclin D1 expression than P-cells. The expression of p53 and p21 also increased in HPV (+) R-cells. The expression of p16 decreased in UMSCC47 R-cells, but was stationary in UDSCC2 R-cell.

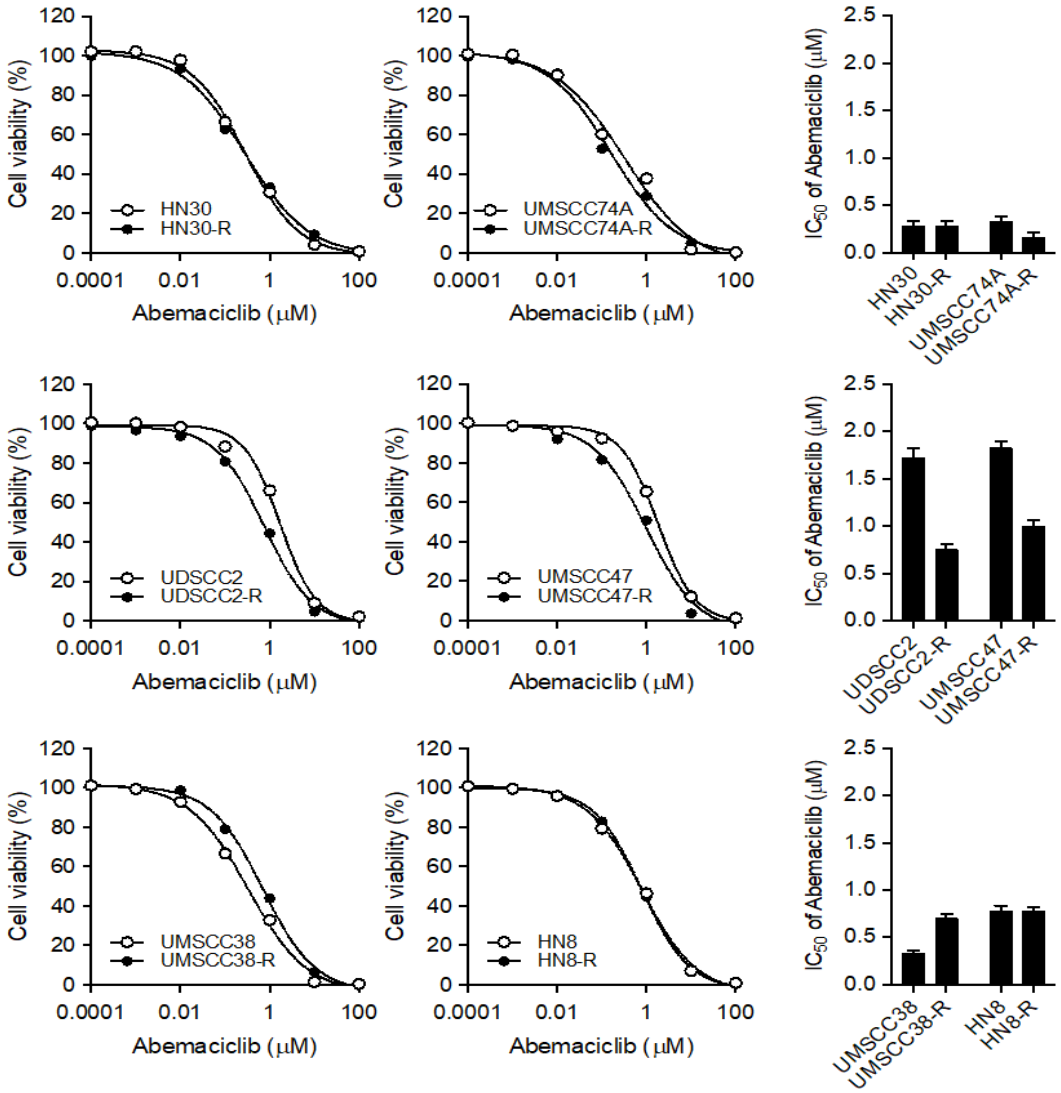

Figure 5. Antitumor effect of abemaciclib monotherapy in P-cells and R-cells

The IC50 of abemaciclib in each cell line was 0.281 (HN30), 0.283 (HN30-R), 0.331 (UMSCC74A), 0.163 (UMSCC74A-R), 1.725 (UDSCC2), 0.752 (UDSCC2-R), 1.828 (UMSCC47), 1.0 (UMSCC47-R), 0.329 (UMSCC38), 0.464 (UMSCC38-R), 0.784 (HN8), and 0.775 (HN8-R).

HPV (+) R-cell showed lower cell viability and IC50 than P-cells, which suggests that abemaciclib had a better antitumor effect in HPV (+) R-cells than P-cells.

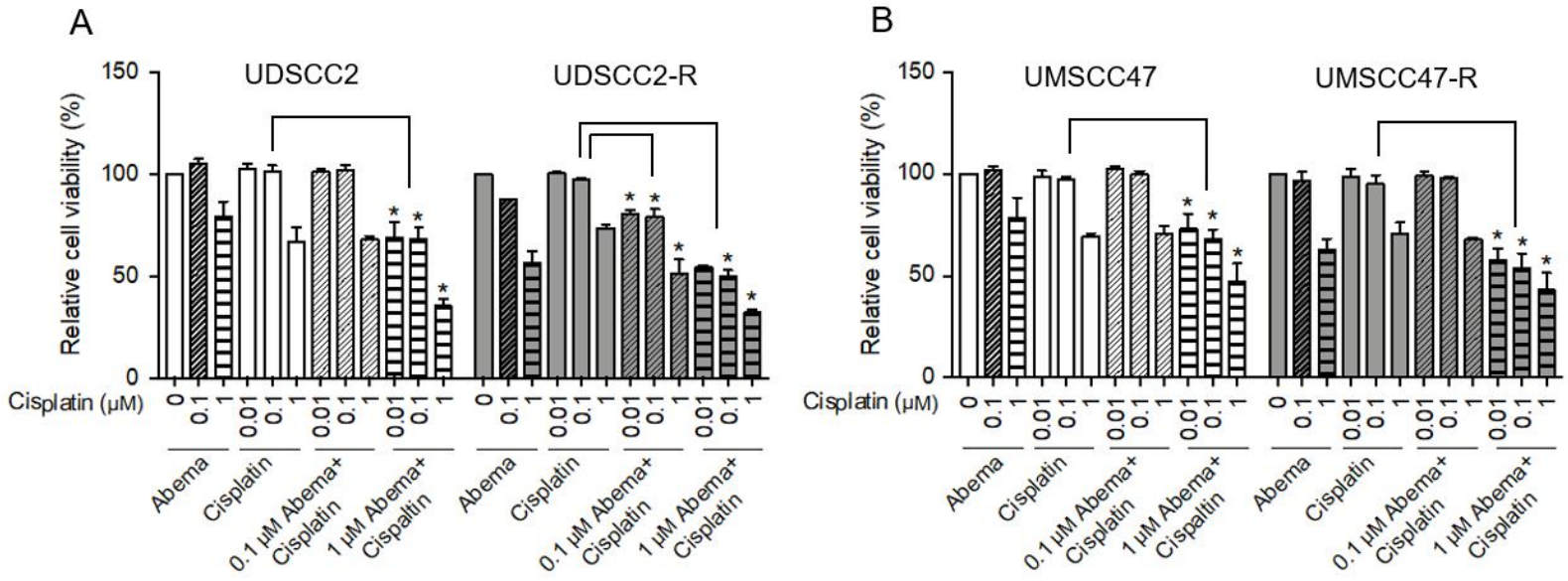

Figure 6. Additive antitumor effect of abemaciclib combined with cisplatin in HPV (+) cell lines

A. In UDSCC2 P-cell, low-dose (0.1 μM) abemaciclib combined with cisplatin had no additive effect. High-dose (1 μM) abemaciclib combination therapy displayed an additive antitumor effect compared with cisplatin monotherapy. In UDSCC2 R-cell, both doses of abemaciclib combination therapy had additional effect, with a dose-dependent effect observed.

B. In both UMSCC47 P-cell and R-cell, low-dose abemaciclib had no additive effect. High-dose abemaciclib combined with cisplatin had an additional effect compared with cisplatin monotherapy. In high-dose abemaciclib, UMSCC46 R-cell had lower cell viability than P-cells.

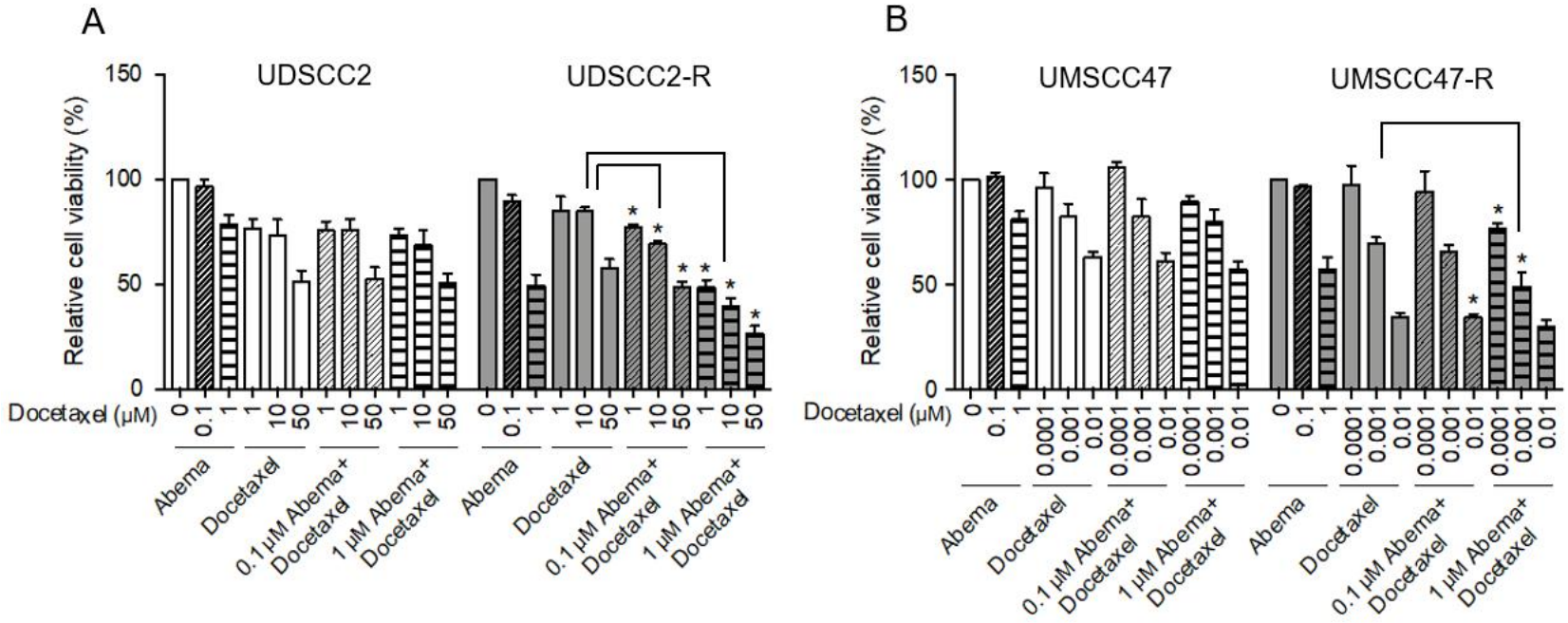

Figure 7. Additive antitumor effect of abemaciclib combined with docetaxel in HPV (+) cell lines

A. In UDSCC2 P-cell, abemaciclib combined with docetaxel had no additive effect compared with docetaxel monotherapy. In UDSCC2 R-cell, both low-dose and high-dose abemaciclib combination therapy showed an additive effect, with a dose-dependent effect observed.

C. In UDSCC47 P-cell, abemaciclib combination therapy with docetaxel showed no additive effect compared with docetaxel monotherapy. In UDSCC47 R-cell, only high-dose abemaciclib showed an additive effect.

Discussion

Even for the advanced stage of HPV (+) cancer, the 5-year overall survival rate ranges from 75% to 80% with radiation-based therapy.[23] Despite the notable treatment outcome of HPV (+) compared to HPV (-), radiation-resistant HPV (+) HNSCC exists. No specific treatment is recommended for recurrent/persistent HPV (+) HNSCC. [17,24] Thus, many HPV (+) HNSCC studies focused on the modification of the HPV (-) HNSCC target agent. Some studies, similar to the clinical trial of cetuximab based de-intensified regimen[25], attempted to identify the target agent for HPV (+) HNPCC; however, a breakthrough has not been achieved.

Abemaciclib is a CKD4/6 inhibitor that has a higher specificity to CDK4 than CDK6. The potential treatment effect of abemaciclib in HNSCC was preclinically proven in large series of patient-derived xenograft models.[26] Further, a clinical study is ongoing to determine the effect of abemaciclib in recurrent and metastatic HNSCC patients resistant to platinum-based treatment (NCT03356587). However, the effect of abemaciclib on HPV (+) HNSCC is thought to be very low due to low Rb expression. The presence of Rb is crucial for the therapeutic effects of CDK4/6 inhibitors and constitutes an already proven biomarker in other tumor entities.[27] HPV (+) HNSCC has functionally inactivated Rb due to degradation by HPV oncoprotein E7 and increased p16, which is a natural suppressor of CDK4/6 complex. Thus, the therapeutic efficacy of CDK4/6 inhibitors against HPV (+) cancer is quite limited.

In this study, we find that HPV (+) R-cells have higher expression of Rb, p-Rb, and cyclin D1 than HPV (+) P-cells. Although the mechanism of Rb expression change in R-cells was not clarified, it may serve as a type of cell defense mechanism against radiation. Radioresistant HPV (+) cells might reactivate an alternate pRb-related carcinogenesis compared to corresponding radiation-susceptible cells. Such finding suggests that pRb-preserved HPV (+) cells would respond to cell cycle inhibitory treatment in the same manner as pRb-preserved HPV (-) cells.

The differences in Rb/p-Rb expression could have a close correlation with the abemaciclib effect. In this study, abemaciclib monotherapy had better antitumor effect in HPV (+) R-cells than HPV (+) P-cells. Abemaciclib combined with cisplatin and docetaxel had an additive effect in P-cells and R-cells. Further, R-cells showed more dramatic response to abemaciclib monotherapy and combination therapy than P-cells. In UDSCC2-R, combination therapy showed dose-dependent response. In conclusion, abemaciclib combination therapy could be applied as a new treatment option for radioresistant HPV (+) HNSCC.

This study had several limitations. First, this study was an *in-vitro* study. Further *in-vivo* studies and clinical trials are needed to confirm the definitive effect of Abemaciclib in HPV (+) HNSCC. Second, the increase in Rb expression in R-cell might be a defense mechanism against radiation; however, the specific mechanism is not clear. The function of the E7 protein that degrades Rb may have changed in R-cells; however, it was not

identified in this study. Further research on abemaciclib for radioresistant HPV (+) HNSCC should be actively conducted.

Conclusion

Radioresistant HPV (+) cell lines had higher expression of Rb, p-Rb, and cyclin D1 than primary HPV (+) cell lines. Both abemaciclib monotherapy and combination therapy had better antitumor effect in radioresistant HPV (+) cell lines than primary HPV (+) cell lines. Abemaciclib may be another treatment option for radioresistant HPV (+) HNSCC.

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국문요약

배경 및 목적

인간유두종바이러스 관련 두경부 편평상피세포암의 빈도가 증가함에 따라, 그 임상적 중요성도 커지고 있다. 인간유두종바이러스 (HPV) 양성 두경부 편평상피세포암은 음성 종양에 비해 방사선 치료에 더 좋은 결과를 보이나, 그럼에도 방사선 저항성 HPV 양성 두경부암 증례들이 꾸준히 보고되고 있다. Abemaciclib 은 싸이클린 의존성 키나아제 4/6 의 선택적 억제제로, HPV 음성 두경부 상피세포암에서 그 효과가 알려져 있으나, 현재까지 HPV 양성 두경부 상피세포암에서는 추천되지 않았다. 방사선 저항성 HPV 양성 두경부 상피세포암에서는 기존의 Rb 단백질의 발현 및 발병 기전이 변화할 것이고, 이에 따라 abemaciclib 에 대한 치료 효과도 달라질 수 있을 것이라 가정하였다. 이번 연구에서는, 방사선 저항성 HPV 양성 두경부암에서 Rb 관련 발병기전의 변화를 확인하고, abemaciclib 의 치료 효과를 평가하였다.

방법

이 연구는 체외 연구로, HPV 감염여부와 p53 유전자 변이여부가 다양한 총 6 가지 세포주 (HN30, UMSCC74A, UDSCC2, UMSCC47, UMSCC38, and HN8)를 대상으로 하였다. 연속적 분할 방사선 조사법을 이용하여 각각의 세포주에 대응하는 방사선 저항성 세포주를 만들었다. 각각의 세포주에서 western blot 을 이용하여 세포 주기 관련 단백질들 (Rb, p-Rb, p53, p21, p16, and Cyclin D1)의 발현

정도를 확인하였다. Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan)를 이용하여, 각각의 세포주에서 abemaciclib 의 단독치료 및 기존 항암제와의 병용요법의 항암효과를 확인하였다.

결과

HPV 양성 방사선 저항성 세포주들은 기존 세포주들에 비해 더 높은 Rb, p-Rb, cyclin D1 발현을 보였다. 반면 HPV 음성 세포주에서는 세포 주기 조절 단백질의 유의한 차이를 보이지 않았다. Abemaciclib 단독 치료하였을 경우, HPV 양성 방사선 저항성 세포주는 기존 세포주에 비해 더 좋은 효과를 보였다. 기존 항암제(cisplatin, docetaxel)와 abemaciclib 의 병합요법 연구에서도 HPV 양성 방사선 저항성 세포주가 기존세포주에 비해 더 나은 효과를 보였다. 이러한 치료효과의 차이는 Rb 및 다른 세포주기 조절 단백질의 변화와 관련이 있을 것으로 생각된다.

결론

HPV 양성 방사선 저항성 세포주들은 기존 세포주에 비해 높은 Rb, p-Rb, cyclin D1 발현을 보였다. Abemaciclib 단독 치료 및 기존 항암제와 병용 치료 모두 HPV 양성 방사선 저항성 세포주에서 더 좋은 항암 효과를 보였다. Abemaciclib 은 방사선 저항성 HPV 양성 두경부 편평상피세포암에서 새로운 잠재적인 치료법으로 고려될 수 있다.