Resveratrol-induced *REG III* expression enhances chemo- and radiosensitivity in head and neck cancer in xenograft mice

SHINJI MIKAMI[1,2], ICHIRO OTA[1], TAKASHI MASUI[1], TOMOKO UCHIYAMA[3], HIDEYUKI OKAMOTO[4], TAKAHIRO KIMURA[1], SHIN TAKASAWA[3] and TADASHI KITAHARA[1]

[1]Department of Otolaryngology-Head and Neck Surgery, Nara Medical University, Kashihara, Nara 634-8522; [2]Department of Otolaryngology-Head and Neck Surgery, Bell Land General Hospital, Higashiyama, Naka-ku, Sakai, Osaka 599-8247; [3]Department of Biochemistry, Nara Medical University, Kashihara, Nara 634-8522; [4]Department of Otolaryngology, Nara City Hospital, Nara, Nara 630-8305, Japan

Received October 31, 2018; Accepted April 23, 2019

DOI: 10.3892/or.2019.7137

Abstract. Identifying the key molecules that enhance chemo- and radiosensitivity in head and neck squamous cell carcinoma (HNSCC) as well as reliable biomarkers for predicting recurrence and metastasis would be desirable to improve the prognosis of HNSCC. Previously, we have reported that *Regenerating gene III* (*REG III*) expression was associated with an improved survival rate for patients with HNSCC. In addition, resveratrol (3,4',5-trihydroxystilbene) significantly increased *REG III* expression in HNSCC cells, and significantly inhibited cell growth, enhanced chemo- and radiosensitivity, and blocked the cancer invasion of HNSCC cells *in vitro*. In the present study, the effect of resveratrol on cancer progression in HNSCC was investigated *in vivo* using a xenograft nude mouse model. The results revealed that resveratrol increased the mRNA level of *REG III in vivo*, which was in agreement with our previous *in vitro* findings. Furthermore, *REG III* increased the antitumor effect of radiation or cisplatin *in vivo*, and resveratrol sensitized HNSCC to irradiation and cisplatin *in vivo*. These results indicated that resveratrol could increase the efficacy of cisplatin and irradiation through the REG III expression pathway, resulting in the inhibition of HNSCC progression *in vivo*.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide (1,2). Cancer in the head and neck region can arise from several functional disorders associated chewing, speech, swallowing and respiration. Therefore, therapy for HNSCC is required to not only cure the disease, but to also preserve the function of the affected area in order to maintain the quality of life of patients with HNSCC. Despite the recent advancements in surgery, chemotherapy and radiotherapy, limited improvements in treating metastatic HNSCC have been achieved (3,4). Two-thirds of all patients present advanced stage III or IV tumors with low locoregional control rates and the long-term survival of patients with HNSCC has remained insufficient (5,6). Therefore, novel agents for potential alternative HNSCC treatments with greater efficacy are urgently required. The present study focused on the human regenerating gene (*REG*) as a reliable biomarker for predicting HNSCC progression. *REG* was first identified in regenerating pancreatic islets in studies on diabetology in 1988 (7). REG family proteins are classified into 4 subfamilies: Types I, II, III and IV. The human *REG* family consists of 5 members: *REG Iα*, *REGIβ*, *REG III*, *hepatocarcinoma-intestine-pancreas/pancreatitis-associated-protein* and *REG IV* (8-13). The *REG* family of proteins has been revealed to serve roles in normal tissue regeneration (14,15) and also the progression of various types of cancers, including esophageal, gastric, lung, liver, colorectal and prostate cancer (16-25). Recently, we reported that *REG III* expression was associated with improved survival rates for HNSCC, and that *REG III* enhanced chemo- and radiosensitivity *in vitro* (26). In addition, as *REG III* contributes to the improvement of the prognosis of HNSCC, in our previous study we searched for the substance that induced the expression of *REG III* and finally revealed that resveratrol (3,4',5-trihydroxy-*trans*-stilbene) significantly increased *REG III* expression in HNSCC cells, and also significantly inhibited cell growth, enhanced chemo- and radiosensitivity, and blocked HNSCC cell invasion *in vitro* (27). The aim of the present study was to investigate the effect of resveratrol on cancer progression in HNSCC *in vivo* for clinical application.

Materials and methods

Cell culture and reagent. Human hypopharyngeal cell carcinoma FaDu cells were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and grown

Correspondence to: Dr Ichiro Ota, Department of Otolaryngology-Head and Neck Surgery, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan
E-mail: iota@naramed-u.ac.jp

Key words: resveratrol, REG family, head and neck squamous cell carcinoma, chemo- and radiosensitivity, xenograft nude mouse

and maintained in Dulbecco's modified Eagle's medium (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and supplemented with 100 U/ml penicillin G and 100 μg/ml streptomycin and 250 ng/ml amphotericin B (Antibiotic-Antimycotic; Gibco; Thermo Fisher Scientific, Inc.). The cells were incubated in 5% CO_2/95% air with a humidified atmosphere at 37˚C. 3,4',5-Trihydroxy-*trans*-stilbene (resveratrol) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Resveratrol was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and then diluted with normal saline to achieve the correct dose in 300 μl of 2% DMSO.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated using a RNAprotect® Cell Mini kit (Qiagen GmbH, Hilden, Germany) from FaDu cells. cDNA was reverse-transcribed from 0.5-2 μg samples of total RNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) as previously described (26-33). cDNA was subjected to RT-PCR with the following primers, which were synthesized and prepared by NGRL (Sendai, Japan): β-actin (NM_001101) sense, 5'-GCGAGAAGATGACCCAGA-3' and antisense, 5'-CAG AGGCGTACAGGGATA-3'; and *REG III* (AB161037) sense 5'-GAATATTCTCCCCAAACTG-3' and antisense, 5'-GAG AAAAGCCTGAAATGAAG-3'.

RT-qPCR was performed using the KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems; Roche Diagnostics, Indianapolis, IN, USA) and Thermal Cycler Dice Real-Time System (Takara Bio, Inc., Otsu, Japan) as previously described (25-33). qPCR was performed with the following thermocycling conditions: An initial step of 3 min at 95˚C followed by 40 cycles of 3 sec at 95˚C and 20 sec at 60˚C. The level of the target mRNA was normalized to the mRNA level of *β-actin* as an internal standard.

Animals. BALB/c nude male mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). All protocols were approved by the Animal Care and Use Committee of Nara Medical University (Nara, Japan). Each cage housed 3 mice with food and water available *ad libitum* in a pathogen-free environment with a 12-h light/dark cycle.

In vivo model for resveratrol-induced REG III expression in HNSCC cells. A total of 6 BALB-c nude male mice were used in each experiment. FaDu cells ($1x10^6$ cells/100 μl saline) were implanted subcutaneously into the right flanks of BALB/c nude male mice (4-5 weeks old). When the tumors reached ~100 mm^3 volume, the mice were randomly assigned into 2 groups (day 0): Vehicle control (normal saline and 2% DMSO) and resveratrol groups. Mice were treated intraperitoneally with resveratrol (100 mg/kg/day) or vehicle until the completion of the experiment. At 30 days following treatment, mice were sacrificed with intraperitoneal administration of pentobarbital (100 mg/kg) and the tumor tissues were harvested. The mRNA levels of intrinsic *REG III* were then examined in tumor tissues via RT-qPCR.

In vivo model of REG III-induced chemo- and radiosensitivity. A total of 12 BALB-c nude male mice (4-5 weeks old) were used to establish a xenograft model in each experiment. FaDu cells transfected with the *REG III* expression plasmid (FaDu-REG III cells) or the neomycin-resistance gene alone (FaDu-mock cells) were used as previously described (26). FaDu-REG III or FaDu-mock cells ($1x10^6$ cells in 100 μl volume) were implanted subcutaneously in the right flanks of BALB/c nude male mice (4-5 weeks old). When the tumor sizes reached ~100 mm^3, the mice were randomly assigned to the treatment groups (n=3/group; day 0). For chemosensitivity experiments, the treatment groups consisted of the vehicle control (normal saline) and cisplatin. On day 0, cisplatin (Nihon Kayaku Co., Tokyo, Japan; 4 mg/kg/week) or normal saline was administered intraperitoneally; a total of 4 injections of cisplatin were administered. For the radiosensitivity experiments, the treatment groups consisted of vehicle control (normal saline) and 6 Gy irradiation. Mice were exposed to radiation using a MBR-1520R system (Hitachi, Ltd., Tokyo, Japan) operated at 150 kV and 20 mA as previously described (27), which delivered the dose at 0.8 Gy/min. For both the chemo- and radiosensitivity experiments, the tumor size was assessed every 3 days. The tumor volume was calculated using the following formula: Volume=$[Lx(W)^2]/2$, where L is the length and W is the width. At 24 days post-treatment, the mice were sacrificed with intraperitoneal administration of pentobarbital (100 mg/kg) and the tumor tissues were harvested.

In vivo model for resveratrol-induced chemo- and radiosensitivity. A total of 12 BALB-c nude male mice were used in each study. FaDu cells ($1x10^6$ cells/100 μl saline) were implanted subcutaneously in the right flanks of BALB/c nude male mice (4-5 weeks old). When the tumors reached ~100 mm^3 volume, the mice were randomly assigned into the following treatment groups (n=3/group; day 0). For the chemosensitivity experiments, the treatment groups consisted of the vehicle control (normal saline and 2% DMSO), vehicle and cisplatin, resveratrol alone and cisplatin + resveratrol. On day 0, the mice were treated intraperitoneally with resveratrol or vehicle until the completion of the experiment; resveratrol was administered at doses of 100 mg/kg for 30 consecutive days. Cisplatin (3 mg/kg/week) or normal saline was administered intraperitoneally on day 0; a total of 4 cycles were administered. For the radiosensitivity experiments, the treatment groups consisted of the vehicle control (normal saline and 2% DMSO), vehicle and 6 Gy irradiation, resveratrol (100 mg/kg/day) alone and 6 Gy irradiation + resveratrol (100 mg/kg/day). For both the chemo- and radiosensitivity experiments, the tumor size was measured every 3 days. Following 30 days of treatment, the mice were sacrificed and the tumor tissues were harvested.

Statistical analysis. Data were expressed as the mean ± standard error. Statistically significant differences between groups were determined by Student's t-test using StatMate IV (Abacus Concepts, Piscataway, NJ, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Induction of REG III mRNA by resveratrol in the tumor tissues of a xenograft mouse model. To elucidate the

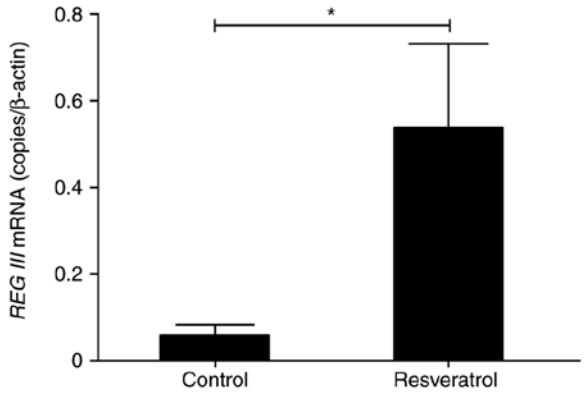

Figure 1. mRNA levels of *REG III* in the tumor tissues of xenograft nude mice treated with vehicle control or resveratrol. Nude male mice were implanted subcutaneously with FaDu cells. Mice were treated intraperitoneally with or without resveratrol. Following 30 days of treatment, tumor tissues were harvested and the mRNA levels of intrinsic *REG III* in tumor tissues were examined by reverse transcription-quantitative polymerase chain reaction. The ratios of the levels of target mRNA normalized to β-actin mRNA are displayed and data are presented as the mean ± standard error of each group (n=3). *P<0.05, as indicated. REG, regenerating gene.

induction of *REG III* in HNSCC cells by resveratrol *in vivo*, the present study investigated the levels of *REG III* mRNA in xenografted FaDu cells with or without resveratrol. The mRNA levels of *REG III* in the resveratrol-treated tumor tissues were significantly increased by resveratrol when compared with the untreated tumor tissues, which were used as the control (Fig. 1). This result was in agreement with the *in vitro* results we previously reported (27). Therefore, resveratrol induced *REG III* mRNA expression *in vivo* as well as *in vitro* in HNSCC.

REG III enhances the efficacy of radiation or cisplatin therapy in a HNSCC xenograft mouse model. To evaluate the effect of *REG III* on chemo- and radiosensitivity in HNSCC *in vivo*, the present study established a HNSCC xenograft model of BALB/c nude mice. An animal xenograft model was generated via injections in nude mice with FaDu cells stably transfected with the *REG III* expression plasmid (FaDu-REG III) or FaDu cells transfected the neomycin-resistance gene alone (FaDu-mock) as the control into the right flank. When tumors reached ~100 mm^3 following 2 weeks, the mice were randomly assigned into 2 groups: Non-treatment, and irradiation or cisplatin therapy groups (day 0), each specific treatment was then applied as aforementioned (Fig. 2A). The efficacy of the radiation or cisplatin therapy was evaluated by monitoring the tumor volume. Regarding the effect of radiation, there were no significant differences in tumor volume between the FaDu-REG III and FaDu-mock in the non-treatment groups. However, in the radiation groups the tumor volume of the FaDu-REG III-treated mice was significantly inhibited when compared with FaDu-mock from day 21 (Fig. 2B-D). Regarding the effect of cisplatin therapy, significant inhibition of tumor progression was observed in the FaDu-REG III group when compared with the FaDu-mock-treated groups from day 15, which corresponded with the results in the radiation groups (Fig. 2B-D). These results indicated that *REG III* enhanced chemo- and radiosensitivity in HNSCC *in vivo* as well as *in vitro,* as previously described (26).

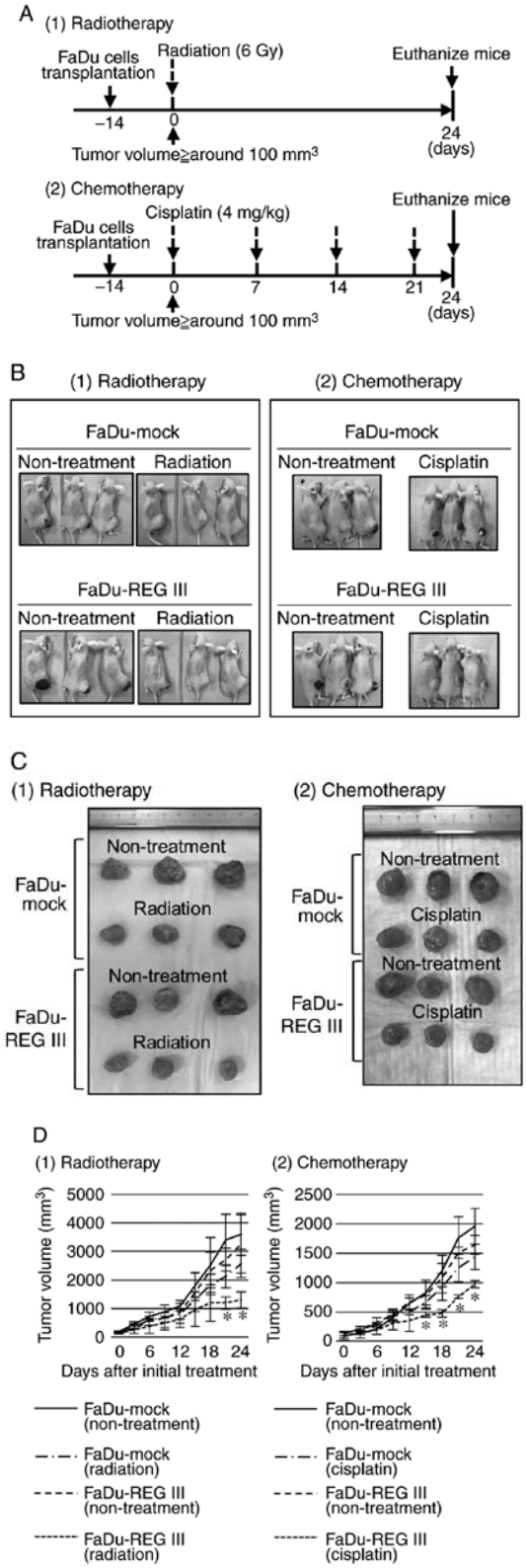

Figure 2. Enhancement of chemo- and radiosensitivity by *REG III* expression in xenograft tumors. A xenograft nude mouse model was generated via the injection of FaDu cells transfected with the *REG III* expression plasmid (FaDu-REG III) or FaDu cells transfected the neomycin-resistance gene alone (FaDu-mock). The mice were randomly assigned into 2 groups: Non-treatment and irradiation therapy (6 Gy), or cisplatin therapy (4 mg/kg/week). (A) The animals were treated as indicated and euthanized on day 24 of treatment. (B) Images of tumor-bearing mice in each group (n=3/group). (C) Images of the excised tumors in each group. (D) The tumor volumes in mice were measured during the course of the experiment (every 3 days) and were calculated using the formula: $V=[Lx(W)^2]/2$. Statistical significance was calculated by Student's t-test. *P<0.05. REG, regenerating gene.

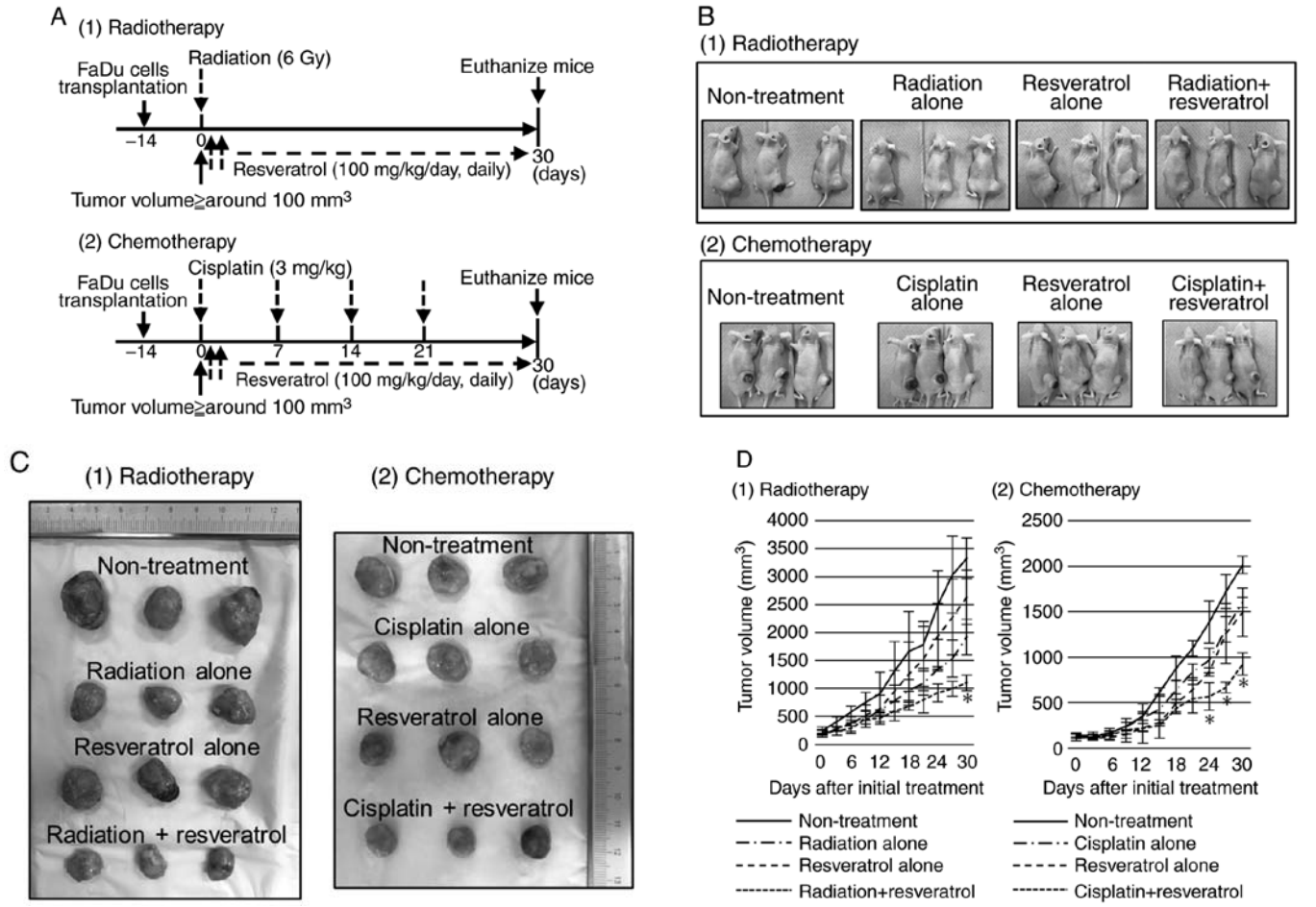

Figure 3. Resveratrol potentiates the effect of irradiation or cisplatin to inhibit the growth of human head and neck squamous cell carcinoma *in vivo*. Xenograft tumors were established by subcutaneous injection of FaDu cells into BALB/c mice. Mice were randomly divided into 4 groups (n=3/group). For radiotherapy experiments, the non-treatment group, radiation (6 Gy) alone group, resveratrol (100 mg/kg/day) alone group, and radiation (6 Gy) with resveratrol (100 mg/kg/day) group were applied. For chemotherapy, the non-treatment group, cisplatin (3 mg/kg/week) alone group, resveratrol (100 mg/kg/day) alone group, and cisplatin (3 mg/kg/week) with resveratrol (100 mg/kg/day) group were used. (A) A schematic representation of the experimental protocol. The animals were euthanized on day 30 of treatment. Images of (B) tumor-bearing mice and (C) excised tumors. (D) The tumor volumes in mice measured during the course of the experiment (every 3 days). Statistical significance was calculated by Student's t-test. $^{*}P<0.05$.

Irradiation or cisplatin therapy with resveratrol synergistically inhibits HNSCC xenograft tumor growth in vivo. To assess the *in vivo* therapeutic potential of resveratrol, the present study examined tumor progression using a HNSCC xenograft model of BALB/c nude mice. Tumors in the right flanks of the mice were established for 2 weeks prior to the initiation of the treatments, then the mice were randomly assigned into 4 groups for experiments evaluating cisplatin therapy or irradiation, respectively (day 0; Fig. 3A). The potential of the treatment was evaluated by assessing the tumor volume. Regarding the potential of radiation therapy, mice in the resveratrol alone group had smaller tumor volumes than mice in the control group; however, the difference was not statistically significant. Mice in the radiation with resveratrol group had significantly smaller tumor volumes than mice in the control group. In addition, the radiation with resveratrol group exhibited significant antitumor effects when compared with the resveratrol alone group, or the radiation alone group on day 30 (Fig. 3B-D). Regarding the potential of cisplatin therapy, mice in the cisplatin alone and cisplatin with resveratrol groups had significantly smaller tumor volumes than mice in the control group. In addition, the cisplatin with resveratrol group exhibited significant antitumor effects when compared with the resveratrol alone or cisplatin alone groups from day 24 (Fig. 3B-D). These results indicated that resveratrol enhanced chemo- and radiosensitivity in HNSCC *in vivo*, which was in agreement with the effects observed with *REG III in vivo*.

Discussion

Despite the progression in the current cancer treatments available, such as surgery, radiation and chemotherapy, these have not been effective in improving the survival rate of HNSCC, particularly hypopharyngeal carcinoma (1-6). Chemo- and radioresistance can cause local recurrence and distant metastasis, which are associated with poor prognosis. Therefore, identification of reliable biomarkers that enhance sensitivity for the chemo- and radiotherapy of HNSCC is highly desirable to improve prognosis. As a biomarker of HNSCC, we have focused on *REG*, whose family of proteins have been associated with diseases such as chronic inflammation and malignant tumors (16-26).

We have previously reported that *REG III* expression was associated with an improved survival rate for patients with

HNSCC (26). In addition, resveratrol significantly increased *REG III* expression, could enhance the chemo- and radiosensitivity, and inhibit cancer progression through the REG III expression pathway in HNSCC cells *in vitro* (27). In the present study, the effect of resveratrol on cancer progression in HNSCC through the *REG III* expression pathway *in vivo* was investigated. The results of the present study demonstrated that resveratrol increased the mRNA level of *REG III in vivo*, which corresponded with our *in vitro* results previously reported (27). This result highlights the potential of resveratrol in inducing the *REG III* expression pathway *in vivo*.

Resveratrol (3,4',5-trihydroxystilbene) is a polyphenolic compound found in grapes and other food products that provides a number of anti-aging health benefits against metabolism, cardiovascular disease and carcinogenesis (34-37). Various previous studies indicated that resveratrol enhanced the sensitivity of chemo- or radiotherapy (38-42). Furthermore, some studies have demonstrated that resveratrol significantly decreased tumor progression (39,40,43). Such *in vitro* studies on the associations between resveratrol and cancer have been reported (34-37,44-50), while, to the best of our knowledge, there are fewer *in vivo* studies.

In the present study, a HNSCC xenograft nude mouse model was established to evaluate the effect of *REG III* on cancer progression in HNSCC *in vivo*. It was demonstrated that *REG III* increased the antitumor effect of radiation or cisplatin *in vivo*. In addition, the *in vivo* therapeutic potential of resveratrol was evaluated, and the results revealed that it significantly sensitized HNSCC to irradiation and cisplatin *in vivo*, although resveratrol is not likely to be a primary treatment for HNSCC. These results indicate that resveratrol has potential for use as an adjuvant anticancer therapy in HNSCC. In our previous studies, similar results were obtained in HSC-4 cells as well as FaDu cells (27). In terms of its effect for HNSCC *in vivo*, further studies whether HSC-4 cells have similar results based on the results of this study are required in the future. Moreover, it is necessary to consider which combination of chemotherapy, radiotherapy and resveratrol is the most effective treatment. The present study performed *in vivo* experiments using a xenograft mouse model. For clinical applications associated with the administration of resveratrol, experiments on an orthotopic transplant mouse model may be required in order to take into consideration the microenvironment in the future. Concerning the bioavailability of resveratrol, recent studies have indicated that resveratrol induces apoptosis or autophagy in several human cancer cell lines and in an animal model of carcinogenesis (49,50). It has been reported that resveratrol induces cell apoptosis and cell cycle arrest via the caspase/cyclin-cyclin dependent kinase (CDK) signaling pathway (49). The present study investigated the anti-carcinogenic potential of resveratrol by analyzing the *REG III* expression pathway. Preliminary experiments were performed to investigate the anti-carcinogenic mechanism of resveratrol, by analyzing the expression of apoptosis-related proteins. These preliminary results revealed that both resveratrol and *REG III* decreased the expression of cyclin D1, B-cell lymphoma-xL and activated caspase-3 compared with the control (data not shown). This indicated that resveratrol may inhibit cancer progression through the REG III pathway via the caspase/cyclin-CDK signaling pathway. However, the underlying mechanism of how resveratrol enhances *REG III*, and how *REG III* enhances the chemo- and radiosensitivity of HNSCC remains unknown. Therefore, further studies are required in the future.

In conclusion, the results of the present study indicate that resveratrol increases the efficacy of cisplatin and irradiation through the *REG III* expression pathway, resulting in the inhibition of tumor growth, in treating HNSCC *in vivo*. The present study provides support for clinical trials using resveratrol as an adjuvant anticancer therapy and may help improve human HNSCC prognosis. However, additional studies will be required to fully define the therapeutic potential of resveratrol for HNSCC.

Acknowledgements

Not applicable.

Funding

The present study was supported in part by Grants-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant nos. 15K10822 and 17K11398).

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

IO, SM and ST conceived and designed the research. SM, TM and TU conducted the experiments. SM, HO, TKim and TU were involved in the analysis and interpretation of data. SM wrote the manuscript. IO and SM revised the manuscript for important intellectual content. TKit and IO were involved in editing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All protocols were approved by the Animal Care and Use Committee of Nara Medical University (Nara, Japan). All procedures performed in the studies were in accordance with the 1964 Declaration of Helsinki and its later amendments.

Patient consent for publication

Not applicable.

Competing interests

The authors state that they have no competing interests.

References

1. Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014.
2. Argiris A, Karamouzis MV, Raben D and Ferris RL: Head and neck cancer. Lancet 371: 1695-1709, 2008.

3. Cooper JS, Zhang Q, Pajak TF, Forastiere AA, Jacobs J, Saxman SB, Kish JA, Kim HE, Cmelak AJ, Rotman M, *et al*: Long-term follow-up of the RTOG 9501/intergroup phase III trial: Postoperative concurrent radiation therapy and chemotherapy in high-risk squamous cell carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 84: 1198-1205, 2012.
4. Adelstein DJ, Saxton JP, Rybicki LA, Esclamado RM, Wood BG, Strome M, Lavertu P, Lorenz RR and Carroll MA: Multiagent concurrent chemoradiotherapy for locoregionally advanced squamous cell head and neck cancer: Mature results from a single institution. J Clin Oncol 24: 1064-1071, 2006.
5. Fung C and Grandis JR: Emerging drugs to treat squamous cell carcinomas of the head and neck. Expert Opin Emerg Drugs 15: 355-373, 2010.
6. Chen YJ, Chang JT, Liao CT, Wang HM, Yen TC, Chiu CC, Lu YC, Li HF and Cheng AJ: Head and neck cancer in the betel quid chewing area: Recent advances in molecular carcinogenesis. Cancer Sci 99: 1507-1514, 2008.
7. Terazono K, Yamamoto H, Takasawa S, Shiga K, Yonemura Y, Tochino Y and Okamoto H: A novel gene activated in regenerating islets. J Biol Chem 263: 2111-2114, 1988.
8. Takasawa S: Regenerating gene (REG) product and its potential clinical usage. Expert Opin Ther Targets 20: 541-550, 2016.
9. Zhao J, Wang J, Wang H and Lai M: Reg proteins and their roles in inflammation and cancer of the human digestive system. Adv Clin Chem 61: 153-173, 2013.
10. Moriizumi S, Watanabe T, Unno M, Nakagawara K, Suzuki Y, Miyashita H, Yonekura H and Okamoto H: Isolation, structural determination and expression of a novel reg gene, human regI beta. Biochim Biophys Acta 1217: 199-202, 1994.
11. Lasserre C, Simon MT, Ishikawa H, Diriong S, Nguyen VC, Christa L, Vernier P and Brechot C: Structural organization and chromosomal localization of a human gene (HIP/PAP) encoding a C-type lectin overexpressed in primary liver cancer. Eur J Biochem 224: 29-38, 1994.
12. Nata K, Liu Y, Xu L, Ikeda T, Akiyama T, Noguchi N, Kawaguchi S, Yamauchi A, Takahashi I, Shervani NJ, *et al*: Molecular cloning, expression and chromosomal localization of a novel human REG family gene, REG III Gene 340: 161-170, 2004.
13. Hartupee JC, Zhang H, Bonaldo MF, Soares MB and Dieckgraefe BK: Isolation and characterization of a cDNA encoding a novel member of the human regenerating protein family: Reg IV. Biochim Biophys Acta 1518: 287-293, 2001.
14. Watanabe T, Yonemura Y, Yonekura H, Suzuki Y, Miyashita H, Sugiyama K, Moriizumi S, Unno M, Tanaka O, Kondo H, *et al*: Pancreatic beta-cell replication and amelioration of surgical diabetes by Reg protein. Proc Natl Acad Sci USA 91: 3589-3592, 1994.
15. Asahara M, Mushiake S, Shimada S, Fukui H, Kinoshita Y, Kawanami C, Watanabe T, Tanaka S, Ichikawa A, Uchiyama Y, *et al*: Reg gene expression is increased in rat gastric enterochromaffin-like cells following water immersion stress. Gastroenterology 111: 45-55, 1996.
16. Macadam RC, Sarela AI, Farmery SM, Robinson PA, Markham AF and Guillou PJ: Death from early colorectal cancer is predicted by the presence of transcripts of the REG gene family. Br J Cancer 83: 188-195, 2000.
17. Harada K, Zen Y, Kanemori Y, Chen TC, Chen MF, Yeh TS, Jan YY, Masuda S, Nimura Y, Takasawa S, *et al*: Human REG I gene is up-regulated in intrahepatic cholangiocarcinoma and its precursor lesions. Hepatology 33: 1036-1042, 2001.
18. Violette S, Festor E, Pandrea-Vasile I, Mitchell V, Adida C, Dussaulx E, Lacorte JM, Chambaz J, Lacasa M and Lesuffleur T: REG IV, a new member of the regenerating gene family, is overexpressed in colorectal carcinomas. Int J Cancer 103: 185-193, 2003.
19. Yonemura Y, Sakurai S, Yamamoto H, Endou Y, Kawamura T, Bandou E, Elnemr A, Sugiyama K, Sasaki T, Akiyama T, *et al*: REG gene expression is associated with the infiltrating growth of gastric carcinoma. Cancer 98: 1394-1400, 2003.
20. Dhar DK, Udagawa J, Ishihara S, Otani H, Kinoshita Y, Takasawa S, Okamoto H, Kubota H, Fujii T, Tachibana M and Nagasue N: Expression of regenerating gene I in gastric adenocarcinomas: Correlation with tumor differentiation status and patient survival. Cancer 100: 1130-1136, 2004.
21. Bishnupuri KS, Luo Q, Murmu N, Houchen CW, Anant S and Dieckgraefe BK: REG IV activates the epidermal growth factor receptor/Akt/AP-1 signaling pathway in colon adenocarcinomas. Gastroenterology 130: 137-149, 2006.
22. Hayashi K, Motoyama S, Koyota S, Koizumi Y, Wang J, Takasawa S, Itaya-Hironaka A, Sakuramoto-Tsuchida S, Maruyama K, Saito H, *et al*: REG I enhances chemo- and radiosensitivity in squamous cell esophageal cancer cells. Cancer Sci 99: 2491-2495, 2008.
23. Hayashi T, Matsubara A, Ohara S, Mita K, Hasegawa Y, Usui T, Arihiro K, Norimura S, Sentani K, Oue N and Yasui W: Immunohistochemical analysis of REG IV in urogenital organs: Frequent expression of REG IV in prostate cancer and potential utility as serum tumor marker. Oncol Rep 21: 95-100, 2009.
24. Zhou L, Zhang R, Wang L, Shen S, Okamoto H, Sugawara A, Xia L, Wang X, Noguchi N, Yoshikawa T, *et al*: Upregulation of REG I alpha accelerates tumor progression in pancreatic cancer with diabetes. Int J Cancer 127: 1795-1803, 2010.
25. Kimura M, Naito H, Tojo T, Itaya-Hironaka A, Dohi Y, Yoshimura M, Nakagawara K, Takasawa S and Taniguchi S: REG Iα gene expression is linked with the poor prognosis of lung adenocarcinoma and squamous cell carcinoma patients via discrete mechanisms. Oncol Rep 30: 2625-2631, 2013.
26. Masui T, Ota I, Itaya-Hironaka A, Takeda M, Kasai T, Yamauchi A, Sakuramoto-Tsuchida S, Mikami S, Yane K, Takasawa S and Hosoi H: Expression of REG III and prognosis in head and neck cancer. Oncol Rep 30: 573-578, 2013.
27. Mikami S, Ota I, Masui T, Itaya-Hironaka A, Shobatake R, Okamoto H, Takasawa S and Kitahara T: Effect of resveratrol on cancer progression through the REG III expression pathway in head and neck cancer cells. Int J Oncol 49: 1553-1560, 2016.
28. Ota H, Tamaki S, Itaya-Hironaka A, Yamauchi A, Sakuramoto-Tsuchida S, Morioka T, Takasawa S and Kimura H: Attenuation of glucose-induced insulin secretion by intermittent hypoxia via down-regulation of CD38. Life Sci 90: 206-211, 2012.
29. Nakagawa K, Takasawa S, Nata K, Yamauchi A, Itaya-Hironaka A, Ota H, Yoshimoto K, Sakuramoto-Tsuchida S, Miyaoka T, Takeda M, *et al*: Prevention of REG I-induced β-cell apoptosis by IL-6/dexamethasone through activation of HGF gene regulation. Biochim Biophys Acta 1833: 2988-2995, 2013.
30. Yamauchi A, Itaya-Hironaka A, Sakuramoto-Tsuchida S, Takeda M, Yoshimoto K, Miyaoka T, Fujimura T, Tsujinaka H, Tsuchida C, Ota H and Takasawa S: Synergistic activations of REG Iα and REG Iβ promoters by IL-6 and glucocorticoids through JAK/STAT pathway in human pancreatic β cells. J Diabetes Res 2015: 173058, 2015.
31. Fujimura T, Fujimoto T, Itaya-Hironaka A, Miyaoka T, Yoshimoto K, Yamauchi A, Sakuramoto-Tsuchida S, Kondo S, Takeda M, Tsujinaka H, *et al*: Interleukin-6/STAT pathway is responsible for the induction of gene expression of REG Iα, a new auto-antigen in Sjögren's syndrome patients, in salivary duct epithelial cells. Biochem Biophys Rep 2: 69-74, 2015.
32. Tsujinaka H, Itaya-Hironaka A, Yamauchi A, Sakuramoto-Tsuchida S, Ota H, Takeda M, Fujimura T, Takasawa S and Ogata N: Human retinal pigment epithelial cell proliferation by the combined stimulation of hydroquinone and advanced glycation end-products via up-regulation of VEGF gene. Biochem Biophys Rep 2: 123-131, 2015.
33. Takasawa S, Kuroki M, Nata K, Noguchi N, Ikeda T, Yamauchi A, Ota H, Itaya-Hironaka A, Sakuramoto-Tsuchida S, Takahashi I, *et al*: A novel ryanodine receptor expressed in pancreatic islets by alternative splicing from type 2 ryanodine receptor gene. Biochem Biophys Res Commun 397: 140-145, 2010.
34. Renaud S and de Lorgeril M: Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 20: 1523-1526, 1992.
35. Frémont L: Biological effects of resveratrol. Life Sci 66: 663-673, 2000.
36. Cal C, Garban H, Jazirehi A, Yeh C, Mizutani Y and Bonavida B: Resveratrol and cancer: Chemoprevention, apoptosis, and chemo-immunosensitizing activities. Curr Med Chem Anticancer Agents 3: 77-93, 2003.
37. Kundu JK and Surh YJ: Cancer chemopreventive and therapeutic potential of resveratrol: Mechanistic perspectives. Cancer Lett 269: 243-261, 2008.
38. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG and Pezzuto JM: Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 10: 218-220, 1997.
39. Bai Y, Mao QQ, Qin J, Zheng XY, Wang YB, Yang K, Shen HF and Xie LP: Resveratrol induces apoptosis and cell cycle arrest of human T24 bladder cancer cells in vitro and inhibits tumor growth in vivo. Cancer Sci 101: 488-493, 2010.

40. Yang S, Li W, Sun H, Wu B, Ji F, Sun T, Chang H, Shen P, Wang Y and Zhou D: Resveratrol elicits anti-colorectal cancer effect by activating miR-34c-KITLG in vitro and in vivo. BMC Cancer 15: 969, 2015.
41. Tan L, Wang W, He G, Kuick RD, Gossner G, Kueck AS, Wahl H, Opipari AW and Liu JR: Resveratrol inhibits ovarian tumor growth in an in vivo mouse model. Cancer 122: 722-729, 2016.
42. Tan Y, Wei X, Zhang W, Wang X, Wang K, Du B and Xiao J: Resveratrol enhances the radiosensitivity of nasopharyngeal carcinoma cells by downregulating E2F1. Oncol Rep 37: 1833-1841, 2017.
43. Yin HT, Tian QZ, Guan L, Zhou Y, Huang XE and Zhang H: In vitro and in vivo evaluation of the antitumor efficiency of resveratrol against lung cancer. Asian Pac J Cancer Prev 14: 1703-1706, 2013.
44. Estrov Z, Shishodia S, Faderl S, Harris D, Van Q, Kantarjian HM, Talpaz M and Aggarwal BB: Resveratrol blocks interleukin-1beta-induced activation of the nuclear transcription factor NF-kappaB, inhibits proliferation, causes S-phase arrest, and induces apoptosis of acute myeloid leukemia cells. Blood 102: 987-995, 2003.
45. Liang YC, Tsai SH, Chen L, Lin-Shiau SY and Lin JK: Resveratrol-induced G2 arrest through the inhibition of CDK7 and p34CDC2 kinases in colon carcinoma HT29 cells. Biochem Pharmacol 65: 1053-1060, 2003.
46. ElAttar TM and Virji AS: Modulating effect of resveratrol and quercetin on oral cancer cell growth and proliferation. Anticancer Drugs 10: 187-193, 1999.
47. Yeh CB, Hsieh MJ, Lin CW, Chiou HL, Lin PY, Chen TY and Yang SF: The antimetastatic effects of resveratrol on hepatocellular carcinoma through the downregulation of a metastasis-associated protease by SP-1 modulation. PLoS One 8: e56661, 2013.
48. Shankar S, Singh G and Srivastava RK: Chemoprevention by resveratrol: Molecular mechanisms and therapeutic potential. Front Biosci 12: 4839-4854, 2007.
49. Liu B, Zhou Z, Zhou W, Liu J, Zhang Q, Xia J, Liu J, Chen N, Li M and Zhu R: Resveratrol inhibits proliferation in human colorectal carcinoma cells by inducing G1/S phase cell cycle arrest and apoptosis through caspase/cyclin CDK pathways. Mol Med Rep 10: 1697-1702, 2014.
50. Wang G, Guo X, Chen H, Lin T, Xu Y, Chen Q, Liu J, Zeng J, Zhang XK and Yao X: A resveratrol analog, phoyunbene B, induces G2/M cell cycle arrest and apoptosis in HepG2 liver cancer cells. Bioorg Med Chem Lett 22: 2114-2118, 2012.