Biochemical and Biophysical Research Communications 392 (2010) 4–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Regenerating gene I regulates interleukin-6 production in squamous esophageal cancer cells

Shuetsu Usami [a,b], Satoru Motoyama [a,b], Souichi Koyota [a], Jingshu Wang [a], Kaori Hayashi-Shibuya [a,b], Kiyotomi Maruyama [b], Naoko Takahashi [b], Hajime Saito [b], Yoshihiro Minamiya [b], Shin Takasawa [c], Jun-ichi Ogawa [b], Toshihiro Sugiyama [a,*]

[a] Department of Biochemistry, Akita University Graduate School of Medicine, Akita, Japan
[b] Department of Surgery, Akita University Graduate School of Medicine, Akita, Japan
[c] Department of Biochemistry, Nara Medical University, Kashihara, Japan

ARTICLE INFO

Article history:
Received 15 December 2009
Available online 7 January 2010

Keywords:
REG I
Interleukin-6
Cytokine
Esophageal cancer

ABSTRACT

Regenerating gene (REG) I plays important roles in cancer cell biology. The purpose of this study was to determine whether REG I affects cytokine production in cancer cells. We transfected TE-5 and TE-9 squamous esophageal cancer cells with REG Iα and Iβ and examined its effects on cytokine expression. We found that transfecting TE-5 and TE-9 cells with REG I Iα and Iβ led to significantly increased expression of interleukin (IL)-6 mRNA and protein, but it had little or no effect on expression of IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17A, interferon-γ, tumor necrosis factor-α, granulocyte-colony stimulating factor or transforming growth factor-β1. The elevated IL-6 expression seen in REG Iα transfectants was silenced by small interfering RNA-mediated knockdown. These finding suggest that REG I may act through IL-6 to exert effects on squamous esophageal cancer cell biology.

Introduction

The human regenerating gene (REG) family belongs to the lectin superfamily and encodes five small, secreted proteins. REG I was originally isolated from pancreatic islet β-cells as an endogenous growth factor [1,2] and was subsequently shown to exert mitogenic effects on both β-cells and gastric stem cells [3,4]. It now appears that REG I plays important roles in tissue regeneration and in cell proliferation and differentiation, as well as in mitogenesis and carcinogenesis in gastric and enteric tissues [5–11]. Among the various functions of REG I, we have been focused on the susceptibility of treatment to provide more appropriate and individualized treatment to patients of esophageal squamous cell carcinoma [12–14].

Increasing evidence suggests that various cytokines play important roles in carcinogenesis and are also involved in mediating invasion, metastasis and apoptosis in many cancers [15,16]. We hypothesized that REG I regulates cytokines, acting through them to exert effects on cancer cell biology. To test that idea, we investigated the effects of REG I on cytokine production in esophageal squamous cancer cells.

Materials and methods

Cell lines and culture. We obtained the TE-5 and TE-9 esophageal squamous cell carcinoma lines from the RIKEN Bio Resource Center, Tsukuba, Japan, and the Cell Resource Center for Biochemical Research Institute of Development, Aging, and Cancer at Tohoku University, Japan. All cells were cultured in RPMI-1640 (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Equitech-Bio, Kerrville, TX) and antibiotics (100 U/ml penicillin G, 100 μg/ml streptomycin, and 2 mg/ml amphotericin B) in a humidified incubator at 37 °C under an atmosphere of 5% CO_2/95% air.

Establishment of stable transfectant for REG I. cDNA fragments encoding human REG Iα or REG Iβ (nucleotides 15–597 of M18963 and nucleotides 58–619 of D16816, respectively) were inserted into the XhoI/XbaI site of the pCI-neo mammalian expression vector (Promega, Madison, WI). The expression vectors or a control vector (without inserted DNA) were then introduced into TE-5 and TE-9 cells by electroporation, after which the cells were cultured for 2 weeks in RPMI-1640 supplemented with 10% FBS and 500 μg/ml Geneticin (Invitrogen, Grand Island, NY). The resulting Geneticin-resistant clones were then harvested (TE-5 REG Iα/TE-5 REG Iβ and TE-9 REG Iα/TE-9 REG Iβ cells) using cloning cylinders, and the expression of REG Iα or REG Iβ protein was confirmed by Western blot analysis.

Immunoblot analysis. Cells were cultured in 10-cm dishes for 24 h, after which serum-free RPMI1640 medium was added, and

* Corresponding author. Address: Department of Biochemistry, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan. Fax: +81 18 884 6443.
E-mail address: sugiyama@med.akita-u.ac.jp (T. Sugiyama).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.12.129

the cells were cultured for an additional 48 h. The supernatant was then collected, and the protein concentration was determined. Samples of extract containing 20 μg of protein were fractionated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose membranes (Trans-Blot Transfer Medium, Bio-Rad Laboratories, Hercules, CA), which were then blocked for 1 h with 5% skim milk in phosphate-buffered saline (PBS) containing 0.1% Tween 20. The membranes were then incubated first with anti-human REG I antibody (1:500 dilution), which we recently purified, overnight at 4 °C, and then with peroxidase-conjugated anti-mouse IgG (diluted 1:1000 in 0.1% Tween 20–PBS; Dako, Glostrup, Denmark) for 1 h. Immunodetection was accomplished using ECL Plus Western blotting detection system (GE Healthcare, Waukesha, WI). The membranes were subsequently exposed to X-ray film.

Real-time RT-PCR assays. For real-time RT-PCR, total RNA was isolated from cells using a TRIzol Reagent (Invitrogen) and PureLink RNA Mini Kit (Invitrogen) according to the manufacturer's instructions. After quantifying the isolated RNA using a spectrophotometer, 2-μg aliquots were reverse transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany).

The primer sequences used to amplify human REG Iα, REG Iβ, interleukin (IL)-6 and β-actin mRNA, and the Universal Probe Library (Roche Diagnostics) number, are shown in Table 1. Real-time PCR was carried out in a LightCycler 480 (Roche Diagnostics) using a LightCycler 480 kit (Roche Diagnostics). Levels of REG Iα, REG Iβ and IL-6 mRNA were normalized to those of β-actin. All experiments were repeated five times per cell line with consistent results ($n = 5$).

Enzyme-linked immunosorbent assays. Cytokine levels in the supernatant of cultured cells were quantified using enzyme-linked immunosorbent assays (ELISAs). Cells (1×10^6) were incubated in 50-ml flasks in RPMI-1640 medium with 10% heat-inactivated FBS for 24, 48 or 72 h at 37 °C, after which the conditioned medium was collected and cleared by centrifugation. The resultant supernatant was then qualitatively assayed for various cytokines [IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17A, interferon (INF)-γ, tumor necrosis factor (TNF)-α, granulocyte-colony stimulating factor (G-CSF), transforming growth factor (TGF)-β1] using a Multi-Analyte Profiler ELISArray Kit (SABiosciences, Frederick, MD) according to manufacturer's instructions.

In addition, IL-6 was quantity assayed using an IL-6 ELISA kit (R&D Systems, Minneapolis, MN). IL-6 measurements were repeated five times per cell line with consistent results ($n = 5$).

REG I knockdown by small interfering RNA. Silencer predesigned small interfering RNA (siRNA) (Ambion, Austin, TX) was introduced into REG Iα-transfected TE-5 and TE-9 cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. In brief, cells were grown to 50% confluence in 6-well plates. For each well, 75 pmol of siRNA targeting REG Iα (ID 217337) or negative control siRNA (Silencer Negative Control #1 siRNA; Ambion) were mixed with 5 μl of Lipofectamine 2000 in 500 μl of Opti-MEM I medium (Invitrogen). The mixture was added to cells in 2 ml of culture medium, after which the cells were incubated for 8 h. The medium was then replaced with fresh culture medium, and the cells were incubated for an additional 72 h. During that period conditioned medium was collected after 24, 48 and 72 h, and assayed for IL-6 using a specific ELISA. In addition, total RNA was isolated from cells, and was used for RT-PCR analysis of IL-6 expression. The siRNA sequences used to this study are shown in Table 2. All experiments were repeated five times per cell line with consistent results ($n = 5$).

Statistical analysis. Data were expressed as the mean ± the standard deviation. Significant differences between two groups were assessed using Student's t test. All analyses were performed using

Table 1
Primer sequences and Universal Probe Library (Roche Diagnostics) numbers used for real-time RT-PCR.

	Primer sequence (5′–3′)	Universal Probe Library
REG Iα	5′-GCTGATCTCCTGCCTGATGT-3′ 5′-CAACTCTGTCTGGGCCTCTT-3′	#21
REG Iβ	5′-CCAACTCGTTCTTCATGCTG-3′ 5′-AGCTCTGTCTGGGACTCCTG-3′	#52
IL-6	5′-GATGAGTACAAAAGTCCTGATCCA-3′ 5′-CTGCAGCCACTGGTTCTGT-3′	#40
β-Actin	5′-CCAACCGCGAGAAGATGA-3′ 5′-TCCATCACGATGCCAGTG-3′	#64

Table 2
siRNA sequence.

		siRNA sequence (5′–3′)
REG Iα	Sense	5′-GCAAUUACAACGGAGUCAATT-3′
	Antisense	5′-UUGACUCCGUUGUAAUUGCTG-3′

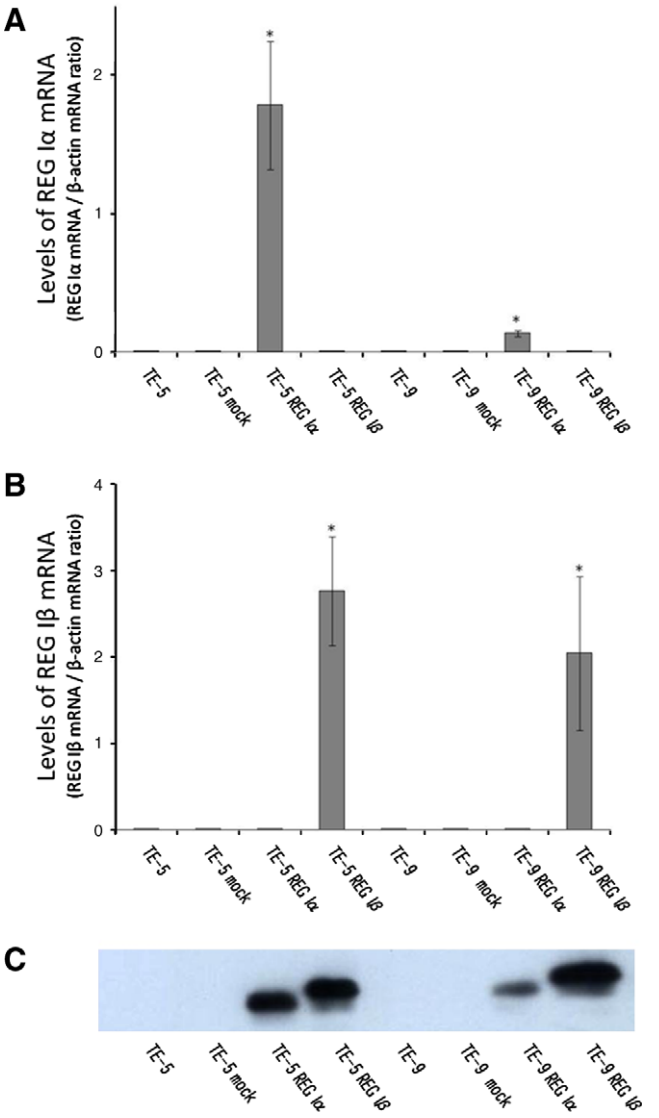

Fig. 1. TE-5 and TE-9 cells transfected with REG Iα and REG Iβ DNA expressed REG Iα (A) and Iβ (B) mRNA and REG I protein (C). * $P < 0.01$ compare to mock cells.

the Stat View J-5.0 (Abacus Concepts, Berkeley, CA), which yielded two-sided *P* values. Values of $P < 0.01$ were considered significant.

Results

Transfection of esophageal cancer cells with REG I

The established REG I transfectants (TE-5 REG Iα/TE-5 REG Iβ and TE-9 REG Iα/TE-9 REG Iβ cells) showed significantly stronger expression of REG I mRNA and protein than the parent cells, or cells transfected with the neomycin-resistance gene alone (mock-transfected) (Fig. 1).

Screening for cytokines secreted by REG I transfectants

We initially tested for the presence of cytokines in culture medium conditioned for 72 h by TE-5 REG Iα/TE-5 REG Iβ or mock-transfected TE-5 cells. Fig. 2 shows a photograph of a representative multi-ELISA plate. Whereas the level of IL-6 in medium from TE-5 REG Iα/TE-5 REG Iβ cells was higher than in that from mock-transfected TE-5 cells, there were no difference between the two cells types with respect to the levels of any of the other cytokines tested (IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17A, INF-γ, TNF-α, G-CSF, TGF-β1).

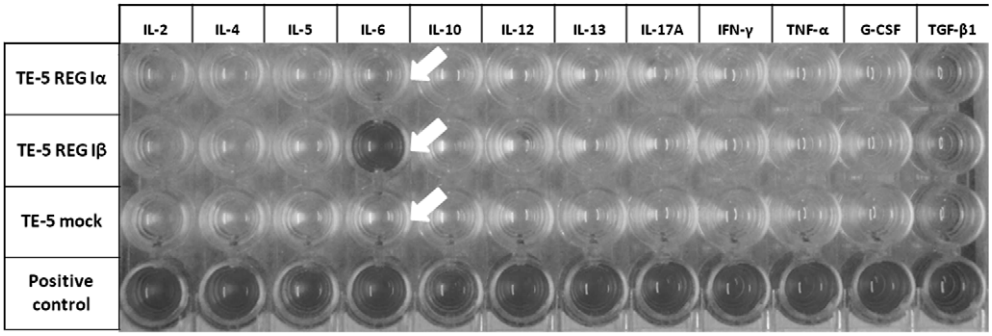

Fig. 2. Cytokine levels in culture supernatant conditioned by REG I transfectants or mock-transfected control cells were screened by a multi-ELISA plate. IL-6 levels were higher supernatants from REG I transfectants than in those from control cells.

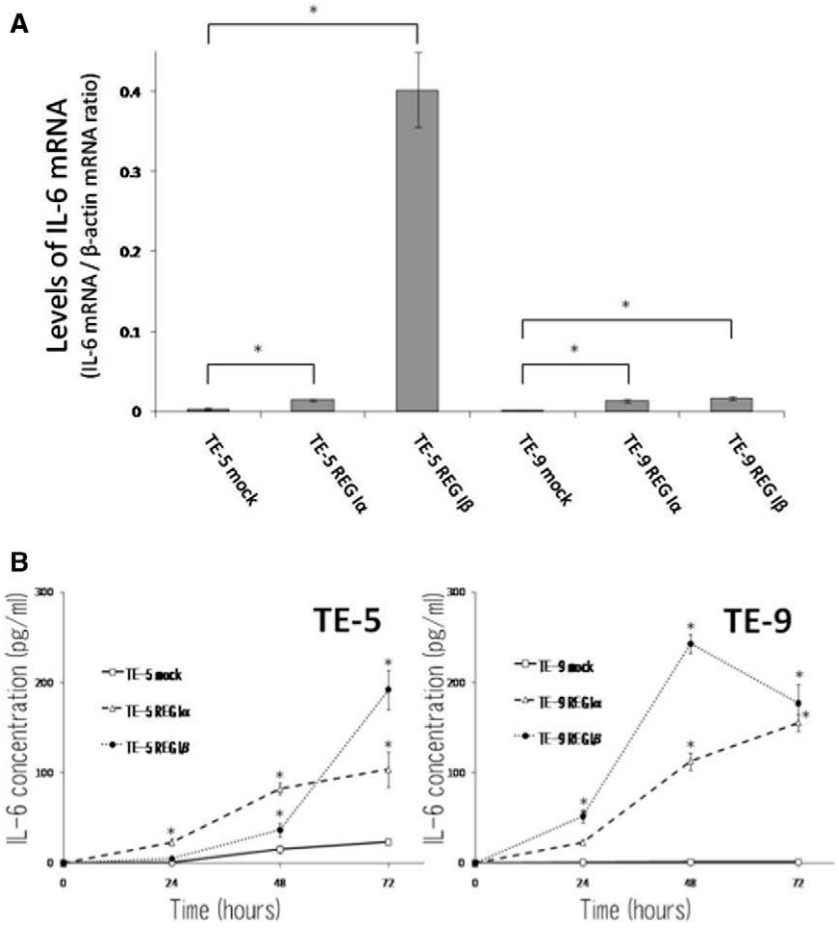

Fig. 3. In RT-PCR assays, REG I transfectants showed significantly stronger expression of IL-6 mRNA than mock-transfected control cells (A). Levels of IL-6 protein in culture supernatants from REG I transfectants and mock-transfected control cells were measured using a specific ELISA (B). * $P < 0.01$ compare to mock cells.

Expression of IL-6 mRNA and protein in REG I transfectants

Expression of IL-6 mRNA was assessed by determining IL-6 mRNA/β-actin mRNA ratio. TE-5 REG Iα/TE-5 REG Iβ and TE-9 REG Iα/TE-9 REG Iβ cells showed significantly stronger expression of IL-6 than mock-transfected TE-5 or TE-9 cells (Fig. 3A). We next determined the IL-6 concentration in culture medium collected from the cells 0, 24, 48 or 72 h after. The time course illustrated in Fig. 3B shows that IL-6 levels increased in a time-dependent manner. Both TE-5 REG Iα/TE-5 REG Iβ and TE-9 REG Iα/TE-9 REG Iβ cells showed significant increased secretion of IL-6 (24, 48 and 72 h), as compared to mock-transfected TE-5 and TE-9 cells.

Effect of REG Iα knockdown on IL-6 production in REG Iα transfectants

Finally, we examined the effect of knocking down REG Iα on expression of IL-6 mRNA and protein in TE-5 and TE-9 cells. We found that siRNA-mediated silencing of REG Iα expression also significantly reduced expression of IL-6 mRNA in REG Iα transfectants (Fig. 4A). Moreover there was a corresponding reduction in the amount of IL-6 protein secreted into the culture medium (Fig. 4B). By contrast, transfecting cells with a negative control siRNA had no effect on expression of IL-6.

Discussion

In the present study, we showed that REG Iα and Iβ transfection increased production of IL-6 mRNA and protein in TE-5 and TE-9 squamous esophageal cancer cells. Conversely, we also showed that silencing REG Iα expression using siRNA diminished expression of IL-6.

IL-6 is a multifunctional cytokine that was originally characterized as a regulator of immune and inflammatory responses; however, elevated expression of IL-6 has also been detected in various epithelial tumors, including esophageal cancer [17–19]. The binding of IL-6 to its receptor leads to activation of JAK family tyrosine kinases, which then stimulate multiple pathways involving MAPKs, PI3Ks, STATs and other signaling proteins [16,20,21]. Moreover, the involvement of IL-6 and its signaling cascades in a variety of cancers is now apparent [22,23], and recent evidence suggests IL-6 is a potential regulator of cancer stem cell activity [24,25]. In that regard, Sansone et al. proposed that IL-6 may act as a positive regulator of tumor stem cell self-renewal in breast cancer, in the same way it regulates epithelial stem cells as part of the natural inflammatory repair program to replace damaged cells [26]. Similarly, Gao et al. provided evidence for the involvement of IL-6 in cancer and identified an EGFR/IL-6/STAT3 signaling cascade involved in the tumorigenesis of non-small cell lung adenocarcinomas [27]. Those findings together with the observation that REG I exerts mitogenic effects on gastric stem cells [3,4] suggest that REG I acts via IL-6 to regulate cancer stem cell activity.

Esophageal squamous cell carcinomas generally have some degree of radiosensitivity, which is why radiotherapy is frequently used to treat both primary and recurrent esophageal cancers [28,29]. Individual tumors can exhibit widely differing susceptibility to radiotherapy, however. Consequently, identification of reliable markers of radiosensitivity or the key molecules and mechanism by which radiosensitivity is enhanced in esophageal cancer cells would be highly desirable and has long been sought.

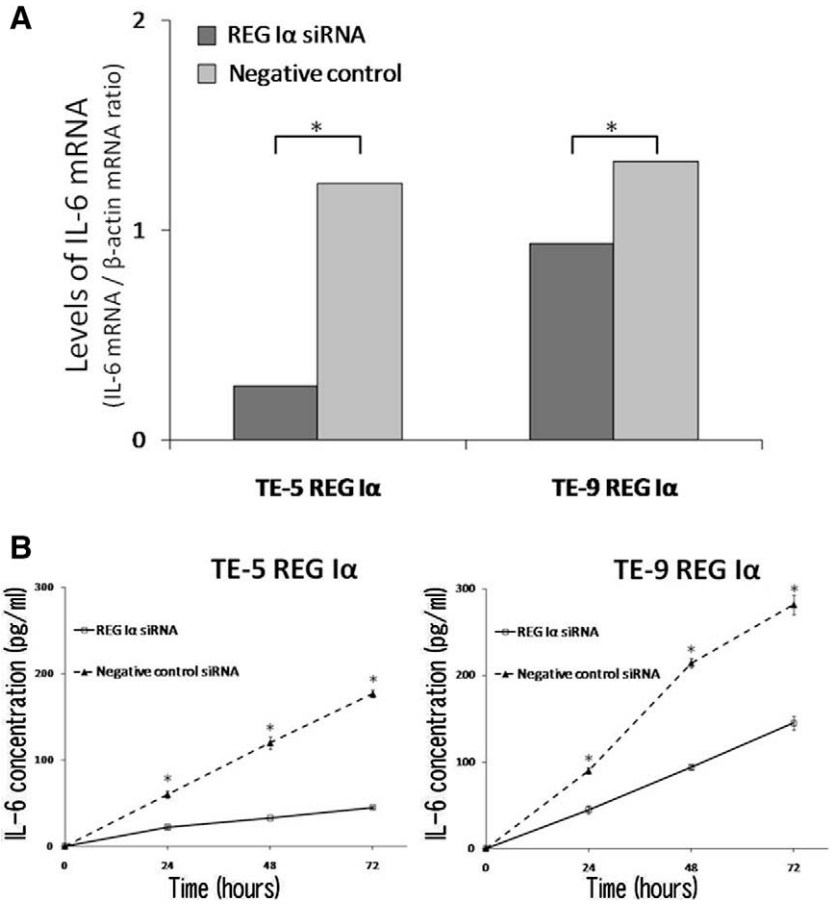

Fig. 4. REG I knockdown reduced the expression of IL-6 mRNA (A) and protein (B) in REG Iα-transfected TE cells. * $P < 0.01$ vs. negative control cells.

We initially reported that REG I transfection enhances the radiosensitivity of cultured esophageal cancer cells [12]. Moreover, we found that, clinically, esophageal squamous cell carcinomas expressing REG I was more susceptible to chemoradiotherapy than those not expressing the molecule [13,14]. In the present study, we found that REG I transfection increased expression of IL-6 mRNA and protein, though IL-6 did not directly enhance radiosensitivity (data were not shown). It was recently reported that IL-6 acts as a radiosensitizer in Kaposi's sarcoma cells [30], and that it enhances the cellular sensitivity to photodynamic therapy in several solid tumors [31–33]. On the other hand, IL-6 has also been reported to exert antiapoptotic and radioprotective effects in esophageal and head and neck squamous cell carcinomas [22,34,35], and it appears the nuclear factor-κB (NF-κB) pathway and/or the STAT1 and STAT3 pathways are involved in mediating the radioprotective effects [36–39]. It thus remains controversial whether IL-6 directly enhances radiosensitivity.

In the pancreatic β-cell regeneration, it has been reported that REG protein acts as a growth factor via its receptor in an autocrine/paracrine fashion [40]. It has been reported that REG Iα gene expression is strongly induced in gastric cancer cells by IL-6 [41], but it also has been reported that IL-6 alone does not induce REG I expression [42]. Whether IL-6 induces REG I expression thus remains unclear; however, we speculate that there is a crosstalk between IL-6 and REG I, and that IL-6 activation by REG I likely feeds back positively to stimulate REG I expression.

In conclusion, we have shown that REG I regulates IL-6 production in an esophageal cancer cell line. This finding suggests that REG I may act through IL-6 to exert effects on squamous esophageal cancer cell biology.

References

[1] K. Terazono, H. Yamamoto, S. Takasawa, et al., A novel gene activated in regenerating islets, J. Biol. Chem. 263 (1988) 2111–2114.
[2] S. Moriizumi, T. Watanabe, M. Unno, et al., Isolation, structural determination and expression of a novel reg gene, human regI beta, Biochim. Biophys. Acta 1217 (1994) 199–202.
[3] M. Unno, T. Itoh, T. Watanabe, et al., Islet beta-cell regeneration and reg genes, Adv. Exp. Med. Biol. 321 (1992) 61–69.
[4] Y. Miyaoka, Y. Kadowaki, S. Ishihara, et al., Transgenic overexpression of Reg protein caused gastric cell proliferation and differentiation along parietal cell and chief cell lineages, Oncogene 23 (2004) 3572–3579.
[5] Y. Yonemura, S. Sakurai, H. Yamamoto, et al., REG gene expression is associated with the infiltrating growth of gastric carcinoma, Cancer 98 (2003) 1394–1400.
[6] H. Fukui, S. Fujii, J. Takeda, et al., Expression of reg1 alpha protein in human gastric cancers, Digestion 69 (2004) 177–184.
[7] T. Ose, Y. Kadowaki, H. Fukuhara, et al., Reg 1-knockout mice reveal its role in regulation of cell growth that is required in generation and maintenance of the villous structure of small intestine, Oncogene 26 (2007) 349–359.
[8] S. Shinozaki, T. Nakamura, M. Imura, et al., Upregulation of REG1α and GW112 in the epithelium of inflamed colonic mucosa, Gut 48 (2001) 623–629.
[9] F.R. Bernard-Perrone, W.P. Renaud, O.M. Guy-Crotte, et al., Expression of REG protein during cell growth and differentiation of two human colon carcinoma cell lines, J. Histochem. Cytochem. 47 (1999) 863–870.
[10] H. Rechreche, G. Montalto, G.V. Mallo, et al., Pap, reg Iα and reg Iβ mRNAs are concomitantly up-regulated during human colorectal carcinogenesis, Int. J. Cancer 81 (1999) 688–694.
[11] D.K. Dhar, J. Udagawa, S. Ishihara, et al., Expression of regenerating gene I in gastric adenocarcinomas: correlation with tumor differentiation status and patient survival, Cancer 100 (2004) 1130–1136.
[12] K. Hayashi, S. Motoyama, S. Koyota, et al., REG I enhances chemo- and radiosensitivity in squamous cell esophageal cancer cells, Cancer Sci. 99 (2008) 2491–2495.
[13] S. Motoyama, T. Sugiyama, Y. Ueno, et al., REG I expression predicts long-term survival among locally advanced thoracic squamous cell esophageal cancer patient treated with neoadjuvant chemoradiotherapy followed by esophagectomy, Ann. Surg. Oncol. 13 (2006) 1724–1731.
[14] K. Hayashi, S. Motoyama, T. Sugiyama, et al., REG Iα is a reliable marker of chemoradiosensitivity in squamous cell esophageal cancer patients, Ann. Surg. Oncol. 15 (2008) 1224–1231.
[15] S. Mocellin, E. Wang, F.M. Marincola, Cytokines and immune response in the tumor microenvironment, J. Immunother. 24 (2001) 392–407.
[16] A. Yoshimura, Signal transduction of inflammatory cytokines and tumor development, Cancer Sci. 97 (2006) 439–447.
[17] T. Kishimoto, Interleukin-6: from basic science to medicine – 40 years in immunology, Annu. Rev. Immunol. 23 (2005) 1–21.
[18] M. Oka, K. Hirose, N. Iizuka, et al., Cytokine mRNA expression patterns in human esophageal cancer cell lines, J. Interferon Cytokine Res. 15 (1995) 1005–1009.
[19] D. Eustace, X. Han, R. Gooding, et al., Interleukin-6 (IL-6) functions as an autocrine growth factor in cervical carcinomas in vitro, Gynecol. Oncol. 50 (1993) 151–159.
[20] K. Imada, W. Leonard, The Jak-STAT pathway, Mol. Immunol. 37 (2000) 1–11.
[21] S.A. Jones, S. Horiuchi, N. Topley, et al., The soluble interleukin 6 receptor: mechanisms of production and implications in disease, FASEB J. 15 (2001) 43–58.
[22] D.S. Hong, L.S. Angelo, R. Kurzrock, Interleukin-6 and its receptor in cancer, Cancer 110 (2007) 1911–1928.
[23] K. Heikkila, S. Ebrahim, D.A. Lawlor, Systematic review of the association between circulating interleukin-6 (IL-6) and cancer, Eur. J. Cancer 44 (2008) 937–945.
[24] T. Hirano, K. Ishihara, M. Hibi, Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors, Oncogene 19 (2000) 2548–2556.
[25] D.R. Hodge, E.M. Hurt, W.L. Farrar, The role of IL-6 and STAT3 in inflammation and cancer, Eur. J. Cancer 41 (2005) 2502–2512.
[26] P. Sansone, G. Storci, S. Tavolari, et al., IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland, J. Clin. Invest. 117 (2007) 3988–4002.
[27] S.P. Gao, K.G. Mark, K. Leslie, et al., Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas, J. Clin. Invest. 117 (2007) 3846–3856.
[28] C. Mariette, G. Piessen, J.P. Triboulet, Therapeutic strategies in oesophageal carcinoma: role of surgery and other modalities, Lancet Oncol. 8 (2007) 545–553.
[29] L. Kleinberg, R.A. Forastiere, Chemoradiation in the management of esophageal cancer, J. Clin. Oncol. 25 (2007) 4110–4117.
[30] A. Hong, M. Zhang, B. Leigh, et al., Induction of interleukin-6 and oncostatin M by radiation in Kaposi's sarcoma cell lines, Int. J. Radiat. Oncol. Biol. Phys. 50 (2001) 533–540.
[31] J. Usuda, T. Okunaka, K. Furukawa, et al., Increased cytotoxic effects of photodynamic therapy in IL-6 gene transfected cells via enhanced apoptosis, Int. J. Cancer 93 (2001) 475–480.
[32] I. Cecic, M. Korbelik, Mediators of peripheral blood neutrophilia induced by photodynamic therapy of solid tumors, Cancer Lett. 183 (2002) 43–51.
[33] H. Du, B.H. Bay, R. Mahendran, et al., Hypericin-mediated photodynamic therapy elicits differential interleukin-6 response in nasopharyngeal cancer, Cancer Lett. 235 (2006) 202–208.
[34] H.D. Schutter, W. Landuyt, E. Verbeken, et al., The prognostic value of the hypoxia markers CA IX and GLUT I and the cytokines VEGF and IL 6 in head and neck squamous cell carcinoma treated by radiotherapy ± chemotherapy, BMC Cancer 5 (2005) 42.
[35] H. Nishino, M. Miyata, K. Kitamura, The effect of interleukin-6 on enhancing the invasiveness of head and neck cancer cells in vitro, Eur. Arch. Otorhinolaryngol. 255 (1998) 468–472.
[36] T. Tamatani, M. Azuma, Y. Ashida, et al., Enhanced radiosensitization and chemosensitization in NF-κB-suppressed human oral cancer cells via the inhibition of γ-irradiation- and 5-FU-induced production of IL-6 and IL-8, Int. J. Cancer 108 (2004) 912–921.
[37] T. Tamatani, M. Azuma, K. Motegi, et al., Cepharanthin-enhanced radiosensitivity through the inhibition of radiation-induced nuclear factor-κB activity in human oral squamous cell carcinoma cells, Int. J. Oncol. 31 (2007) 761–768.
[38] C.M. Leu, F.H. Wong, C. Chang, et al., Interleukin-6 acts an antiapoptotic factor in human esophageal carcinoma cells through the activation of both STAT3 and mitogen-activated protein kinase pathways, Oncogene 22 (2003) 7809–7818.
[39] E.V. Efimova, H. Liang, S.P. Pitroda, et al., Radioresistance of Stat1 over-expressing tumor cells is associated with suppressed apoptotic response to cytotoxic agents and increased IL6–IL8 signalling, Int. J. Radiat. Biol. 85 (2009) 421–431.
[40] H. Okamoto, S. Takasawa, Recent advances in the Okamoto model; the CD38-cyclic ADP-ribose signal system and the regenerating gene protein (Reg)-Reg receptor system in β-cells, Diabetes 51 (2002) 462–473.
[41] A. Sekikawa, H. Fukui, S. Fujii, et al., REG Iα protein may function as a trophic and/or anti-apoptotic factor in the development of gastric cancer, Gastroenterology 128 (2005) 642–653.
[42] T. Akiyama, S. Takasawa, K. Nata, et al., Activation of Reg gene, a gene for insulin-producing β-cell regeneration: poly(ADP-ribose) polymerase binds Reg promoter and regulates the transcription by autopoly(ADP-ribosyl)ation, Proc. Natl. Acad. Sci. USA 98 (2001) 48–53.