Down-regulation of Expression of Interleukin-6 and its Receptor Results in Growth Inhibition of MCF-7 Breast Cancer Cells

XIAN-PENG JIANG, DING CHENG YANG, ROBERT L. ELLIOTT and JONATHAN F. HEAD

Mastology Research Institute, Elliott-Elliott-Head Breast Cancer Research and Treatment Center, Baton Rouge, LA, U.S.A.

Abstract. *Interleukin-6 (IL-6) plays an important role in the neoplastic process through its action on cancer cell adhesion, motility, proliferation, tumor-specific antigen expression, and thrombopoiesis. IL-6 exerts its activity by binding to a high affinity receptor complex consisting of two membrane glycoproteins: the 80 kDa IL-6 a-receptor subunit (IL-6R) and the 130 kDa signal-transducing protein (GP130). In the present study, MCF-7 breast cancer cells were cultured with human IL-6 and IL-6 soluble receptor (sIL-6R). MCF-7 cells were also treated with either antibodies specific to human IL-6 and IL-6R, or synthetic antisense oligodeoxynucleotides (ODNs) targeted to IL-6 and IL-6R genes. Cell growth was measured, and it was found that human IL-6 and sIL-6R did not significantly increase the proliferation of MCF-7 cells. When IL-6 produced by the MCF-7 cells was bound by rabbit anti-human IL-6 antibody, there was a significant dose-dependent inhibition of cell proliferation. IL-6 and IL-6R antisense ODNs caused a marked and specific decrease in IL-6 and IL-6R mRNA and proteins, respectively. Both IL-6 and IL-6R antisense ODNs significantly inhibited the proliferation of MCF-7 cells, but the inhibitory effect of IL-6R antisense ODN was greater than that of IL-6 antisense ODN (IC_{50}: IL-6R: 1 μM; IL-6: 5 μM, 72-hour incubation). Addition of exogenous IL-6 partially reversed the growth inhibition caused by IL-6 antisense ODN but not the growth inhibition caused by IL-6R antisense ODN. In conclusion, IL-6 plays an important role in maintaining the growth of MCF-7 breast cancer cells. These results suggest careful modulation of IL-6 and IL-6R expression of cells as a potential approach for breast cancer therapy.*

Correspondence to: Xian-Peng Jiang, Mastology Research Institute, Elliott-Ellott-Head Breast Cancer Research and Treatment Center, 17050 Medical Center Drive, 4th Floor, Baton Rouge, LA 70816, U.S.A. Tel: +1 2257553070, Fax: +1 2257553085, e-mail: xjiang@eehbreastca.com

Key Words: Interleukin-6, interleukin-6 receptor, breast cancer, MCF-7, antisense, oligodeoxynucleotides.

Interleukin-6 (IL-6) is a multipotent cytokine that plays an important role in immune responses, inflammation, bone metabolism, reproduction, arthritis, aging and neoplasia (1). It has been shown that serum levels of IL-6 are a prognostic factor in various types of cancer (multiple myeloma, Hodgkin's lymphoma, renal cell carcinoma, bladder carcinoma, ovarian cancer, esophageal squamous cell carcinoma, and breast cancer (2-12)). A high concentration of serum IL-6 is associated with aggressive tumor types, and poor disease-free and overall survival. IL-6 is involved in cancer progression through the decrease in cancer cell adhesion and increase in cancer cell motility, induction of thrombopoiesis, increase in cancer cell proliferation, as well as stimulation of tumor-specific antigen expression (13-16). It has also been reported that breast cancer cells treated *in vitro* with IL-6 express higher levels of a breast cancer antigen (CA15-3) and carcinoembryonic antigen (CEA) (16).

IL-6 exerts its activity through binding to a high affinity receptor complex consisting of two membrane glycoproteins: the 80 kDa IL-6 binding receptor protein (IL-6R) and the 130 kDa signal-transducing protein (GP130). IL-6R has a 55 kDa isoform, the soluble IL-6R (sIL-6R). sIL-6R may develop as a result of proteolytic cleavage and release of the membrane-bound form of IL-6R. No naturally occurring mRNA encoding a truncated from of the IL-6R has been reported. IL-6R is not capable of inducing signal transduction directly, but must form a hexameric IL-6: IL-6R:GP130 complex to be fully activated and induce cell signaling. The sIL-6R also forms a fully active hexameric IL-6:sIL-6R:GP130 complex which induces cell signaling (17-19).

In order to further demonstrate the role of IL-6 in the growth of breast cancer cells, we treated MCF-7 cells with specific antibodies that bind IL-6 and IL-6R proteins, and antisense ODNs that inhibit gene expression of IL-6 and IL-6R. Inhibition of cell growth, and inhibition of IL-6 and IL-6R gene expression and protein secretion were determined.

Materials and Methods

Human recombinant IL-6, human recombinant sIL-6R, rabbit polyclonal anti-human IL-6 IgG (IL-6Ab), mouse monoclonal anti-human IL-6R IgG1 (IL-6RAb), and rabbit IgG and mouse IgG1 controls were purchased from Sigma (St. Louis, MO, USA). The human sIL-6R was produced by cloning the cDNA of the IL-6R and introducing a stop codon into the cDNA sequence immediately preceding the transmembrane-bound form of the receptor. The CytElisa™ IL-6 kit and the Pelikine Compact™ soluble human IL-6 receptor ELISA kit were obtained from SYTImmune Science, Inc. (College Park, MD, USA) and Research Diagnostics, Inc. (Flanders, NJ, USA), respectively.

Antisense oligodeoxynucleotides (ODNs). ODNs was synthesized by GeneLab, Louisiana State University (Baton Rouge, LA, USA). All ODNs were completely phosphorothioated and purified by HPLC. The antisense ODN sequence 5'-TCCTGGGGGTACT-3' (IL-6AS) is specific for the second exon of the IL-6 gene (20) and was designed to target *IL-6* mRNA. The antisense ODN sequence 5'-ACGGCCAGCATGC-3' (IL-6RAS) flanking the translation initiation codon of the *IL-6R* cDNA (21) was created to target IL-6R mRNA. Complementary sense ODNs, 5'-AGTACCCCCAGGA-3' (IL-6S) and 5'-GCATGCTGGCCGT-3' (IL-6RS), and random sequence (RS) 5'-ATACACCATACGT-3' were used as controls.

Cell culture. The MCF-7 human breast cancer cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). MCF-7 cells were maintained in alpha modification of Minimum Essential Medium Eagle (αMEM) supplemented with 10% fetal calf serum, 1 mM glutamine and 0.05 mg/ml gentamicin (Life Technologies, Inc., Frederick, MD, USA) in 5% CO_2 at 37˚C. Cells were removed from cell culture flasks or plates by trypsin-EDTA (0.05% trypsin and 0.53 mM EDTA) digestion.

^{3}[H]-Thymidine incorporation assay. Cell growth was measured with a ^{3}[H]-thymidine incorporation assay. MCF-7 cells (2,000 cells/well) were plated into each well of a 96-well cell culture plate and incubated overnight. Culture media were replaced with media containing increasing concentrations of human recombinant IL-6, IL-6R, IL-6Ab, IL-6RAb or ODNs, and the cells were incubated with the agents for 3 days. ^{3}[H]-Thymidine (0.1 μCi/well) was added to each well for the last 16 hours of incubation. The cells were removed from the plate by trypsin-EDTA digestion and harvested onto a glass-fiber filter (Skatron basic96 Harvester, Skatron Inc., Sterling, VA, USA). The radioactivity incorporated into the cell DNA was determined by liquid scintillation counting (LS 6500; Beckman Co., Fullerton, CA, USA). Cell proliferation was quantitated by ^{3}[H]-thymidine incorporation and expressed as a percentage of that of the untreated control. The experiments were repeated three times independently.

Enzyme linked immunosorbent assay (ELISA). ELISA was used to determine the IL-6 and IL-6R secretion by MCF-7 cells into the culture media. MCF-7 cells (2×10^5 cells/well) were plated and cultured overnight in 6-well cell culture plates. Culture media were replaced with media containing ODNs. The cells were cultured for an additional 72 hours in the presence of ODNs. The culture media was collected and stored at −70˚C until IL-6 and IL-6R secretion was quantitated by ELISA. The cells were removed from the plate by trypsin-EDTA digestion, counted with trypan blue staining of viable cells, and pelleted by centrifugation. Cell pellets were used to isolate RNA. The concentrations of IL-6 and IL-6R in the culture media were quantitated by the CytElisa™ IL-6 kit (SYTImmune Science, Inc., College Park, MD, USA) and the Pelikine Compact™ soluble human IL-6 receptor ELISA kit (Research Diagnostics, Inc., Flanders, NJ, USA), respectively, according to the manufacturer's protocol. In brief, the supernatants from MCF-7 cultures were added to wells which were pre-coated with murine monoclonal IL-6 or IL-6R antibodies. IL-6 or IL-6R specific rabbit anti-human polyclonal antibodies were then added to the 96-well plate and incubated for 3 hours at room temperature. After washing, the wells were covered with goat anti-rabbit polyclonal antidody conjugated to alkaline phosphatase and incubated for another 45 minutes at room temperature. After washing again, the color-generating solution was added to the plate and the optical density (OD) of the liquid in each well was measured. The standard curve demonstrated a linear relationship between the OD and the IL-6 and IL-6R concentrations. Using the standard curve, the concentrations of IL-6 and IL-6R in the media were determined. The amount of IL-6 and IL-6R per 1×10^6 MCF-7 cells was calculated. The experiments were repeated three times independently.

Reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR was employed to measure the mRNA levels of IL-6 and IL-6R in MCF-7 cells. Cellular RNA from 2×10^5 to 5×10^5 cells was isolated by the quanidinium isothiocyanate method using a Glass Max RNA Micro Isolation Kit (Life Technologies). The RNA was quantitated by spectrophotometry at a wavelength of 260 nm (Beckman Co.). One microgram of denatured RNA was used for each reverse transcriptase (RT) reaction. The RT mixture included 50 mM Tris-HCI (pH 8.3), 40 mM KCl, 1 mM (DTT), 6 mM MgCl2, 0.5 mM dNTP, 0.1 mM Oligo(dT), 100 μg/ml (BSA) and 0.25 units AMV reverse transcriptase (Life Technologies). The reaction mixture was incubated at 37˚C for 60 min. PCR primers for target and control genes were synthesized by GeneLab, Louisiana State University, Baton Rouge, Louisiana. The primers used for amplification of the fragment of *IL-6* cDNA (628 bp) were 5'-ATGAACTCCTTCTCCA CAAG-3' (upstream) and 5'-AGAGCCCTCAGGCT GGACTG-3' (downstream); the primers for amplification of *IL-6R* (251 bp) were 5'-ATTGCCATTGTTCT GAGGT-3' (upstream) and 5'-TAGTCTG TATTGCTGATGTC-3' (downstream) (22). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as internal standard and the primers used for *GAPDH* were 5'-TGAAGGTCGGAGT CAACGGATTTGGT-3' (upstream) and 5'-CATGTGGGCCATG AGGTCCACCAC-3' (downstream). PCR was performed in a total volume of 25 μl with the primers for *IL-6, IL-6R* and GAPDH. Each PCR cycle included: denaturation at 94˚C for 0.5 minutes, annealing at 65˚C for 0.5 min and extension at 72˚C for 1.5 min in an Ome-E DNA Thermal PCR instrument (National Labnet Co., Woodbridge, NJ, USA). After 35 cycles, there was a final elongation step at 72˚C for 10 min. The PCR products were run on a 2% agarose gel and stained with ethidium bromide, and the band densities were evaluated with Eagle Eye II Still Video System (Stratagene Cloning System, La Jolla, CA, USA). The experiments were repeated three times independently.

Statistics. Differences in proliferation rates, mRNA levels and protein secretion between cells were evaluated for statistical significance by Student's *t*-test. Differences were considered statistically significant when $p \leq 0.05$.

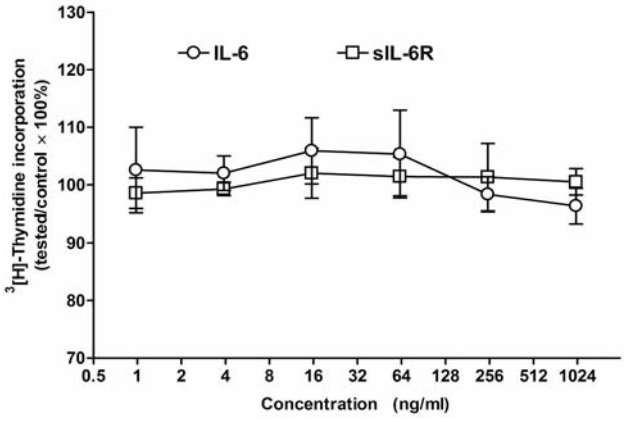

Figure 1. *The proliferation of MCF-7 cells at 72 hours in media with human recombinant IL-6 or sIL-6R. IL6 and sIL-6R had not significant effect on cell proliferation of MCF-7 cells. Values are mean±standard deviation (n=3).*

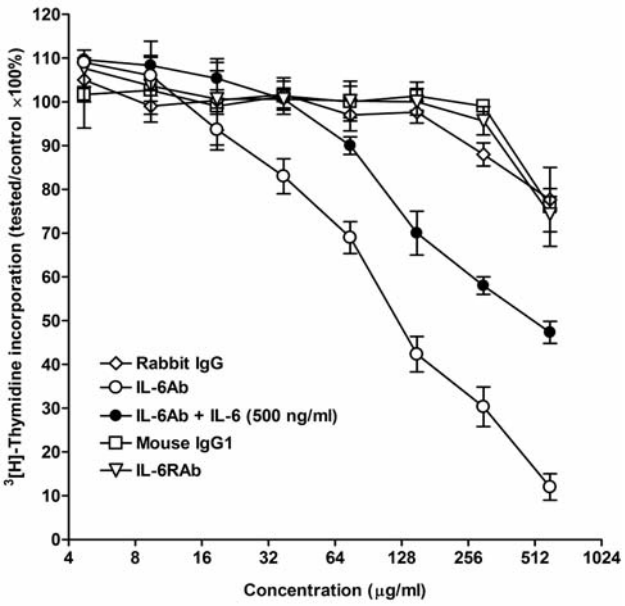

Figure 2. *The growth of MCF-7 breast cancer cells at 72 hours in media with IL-6Ab, IL-6RAb, rabbit IgG or mouse IgG1. The addition of the IL-6 significantly abrogated the cytotoxicity of IL-6Ab. Values are mean±standard deviation (n=3).*

Results

Effects of exogenous IL-6 and IL-6R protein on MCF-7 cells. Previously, investigators have demonstrated in the T47D breast carcinoma cell line that IL-6 simultaneously inhibits cell proliferation and increases cell migration (23). However, we found that human IL-6 had no significant effect on the growth of MCF-7 cells, even when the concentration was increased to 1,000 ng/ml (Figure 1). A slight inhibition of growth of MCF-7 cells with 1,000 ng/ml of IL-6 was observed, but the difference was not statistically significant ($p>0.05$, paired *t*-test, compared to control). In agreement with the findings for IL-6, the sIL-6R also did not affect the growth of MCF-7 cells, even when the concentration was increased to 1,000 ng/ml ($p>0.05$, paired *t*-test, compared to control) (Figure 1).

Growth inhibition of MCF-7 cells caused by specific IL-6Ab. In the present study, MCF-7 cells were incubated with IL-6Ab or IL-6RAb for 72 hours. MCF-7 cells were also cultured in media containing the rabbit IgG and mouse IgG1, the isotype-matching antibody controls. Cell proliferation was determined by ^{3}[H]-thymidine incorporation. High concentrations of rabbit IgG and mouse IgG1 control produced non-specific inhibitory effects on MCF-7 cells. We found that the IL-6Ab inhibited the growth of MCF-7 cells in a dose-dependent manner, and the addition of recombinant human IL-6 (500 ng/ml) abrogated the inhibitory effect. However, the IL-6RAb had no significant effect on the growth of MCF-7 cells. Additionally, the IL-6RAb showed non-specific inhibition, which was the same as that of the mouse IgG1 control (Figure 2).

Effects of antisense ODNs on target mRNA. Figure 3 shows PCR products of total RNA from MCF-7 cells treated with antisense and sense ODNs specific for *IL-6* and *IL-6R*. cDNA was synthesized by the reverse transcription reaction from mRNA templates. *IL-6* and *IL-6R* cDNA were amplified by PCR with specific primers. To ensure that reverse-transcription efficiencies were comparable between test groups, the level of *GAPDH* cDNA (a stable housekeeping gene) was measured using PCR for each reverse-transcription reaction. The 628 bp IL-6 band, 251 bp IL-6R band and 981 bp GAPDH band were seen on the agarose gels (Figures 3A and 3B). mRNA levels of *IL-6* and *IL-6R* were defined as the ratio of *IL-6* or *IL-6R* to *GAPDH*, and were inhibited by antisense *IL-6* and *IL-6R* ODNs, respectively, in a dose-dependent manner (Figures 3 and 4). Although sense IL-6 and IL-6R ODNs slightly reduced mRNA of IL-6 and IL-6R, respectively, the inhibitory effects of sense ODNs were significantly smaller than those of the corresponding antisense ODNs (both $p<0.05$, paired *t*-test, ODNs at 20 μM). The random sequence (RS) ODNs had neither inhibitory nor stimulatory effects on the expression of IL-6 and IL-6R (data not shown).

Inhibition of IL-6 and IL-6R protein secretion by ODNs. MCF-7 cells were treated for 72 h and the concentrations of IL-6 and sIL-6R in the culture media were measured by ELISA. Table I shows that IL-6 and IL-6R sense ODNs at a concentration of 20 μM did not significantly influence the production of IL-6 and sIL-6R, respectively (both $p>0.05$, paired *t*-test), but IL-6 and IL-6R anti-sense ODNs significantly suppressed the secretion of IL-6 and sIL-6R, respectively ($p<0.05$ and $p<0.01$, repectively, paired *t*-test).

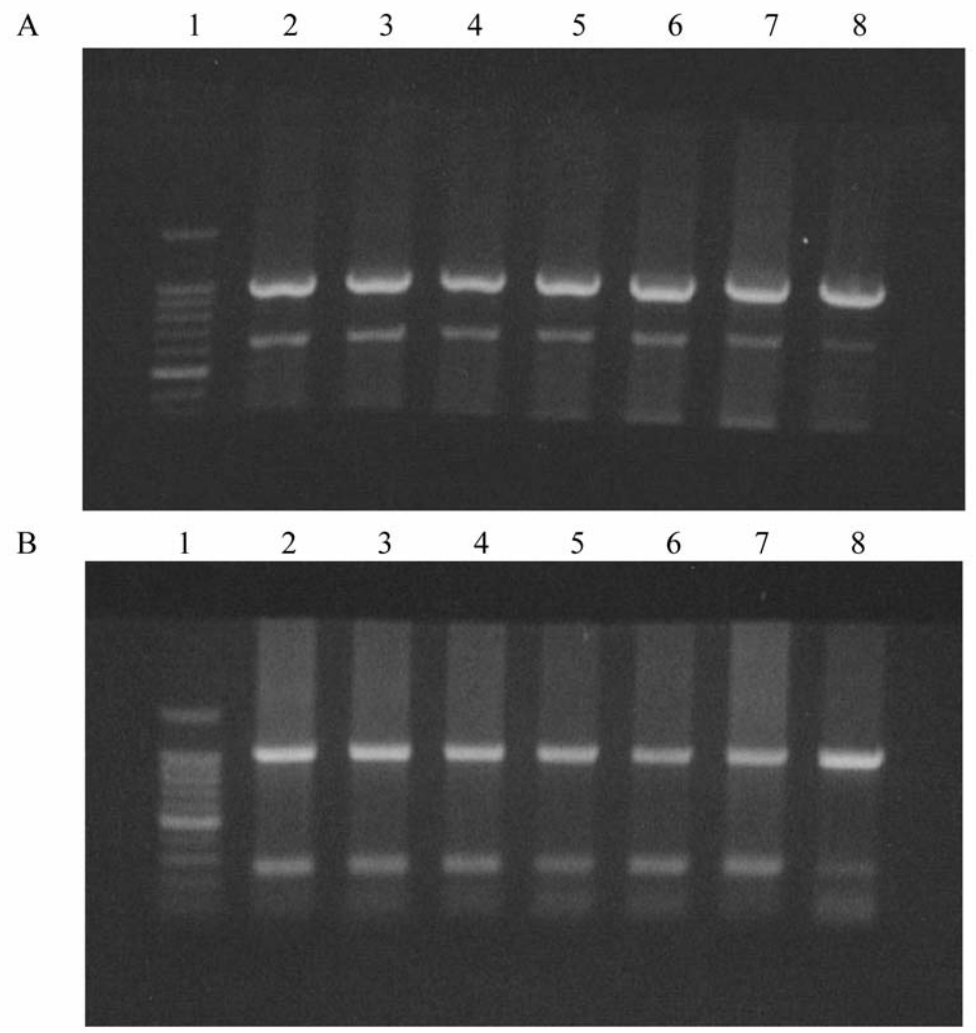

Figure 3. *Agarose gel showing effects of IL-6 and IL-6R ODNs on the expression of A: IL-6 and B: IL-6R mRNA in MCF-7 cells. Cells were treated for 72 h and mRNA was amplified by RT-PCR. The sample lanes are: 1: 100 bp DNA ladder; 2: MCF-7 control; MCF-7 cells treated with 3: 1.25 μM, 4: 5 μM and 5: 20 μM of sense ODN; and 6: 1.25 μM, 7: 5 μM and 8: 20 μM antisense ODN, respectively.*

The RS ODN had no effect on the production of IL-6 and IL-6R in MCF-7 cells (data not shown).

Effects of IL-6 and IL-6R anti-sense ODNs on the proliferation of MCF-7 cells. MCF-7 cells were incubated in media containing ODNs for 72 h and the proliferation was measured by ^{3}[H]-thymidine incorporation. We found IL-6 and IL-6R anti-sense ODNs inhibited the growth of MCF-7 cells in a dose-dependent manner (Figure 5). The IC_{50} of IL-6RAS ODN was significantly lower than that for the other ODNs. The IC_{50}s of IL-6S and IL-6RS ODNs were significantly higher than those of the corresponding anti-sense ODNs (Table II). In addition, we found recombinant human IL-6 was able to partially abrogate the cytotoxicity of IL-6AS but not IL-6RAS ODN (Figure 6).

Discussion

Depending on the target cells, IL-6 induces various and sometimes contrasting biological responses. IL-6 promotes cell proliferation of prostate carcinoma (24), colon carcinoma (25), leukemia (26), melanoma (27), and renal cell carcinoma (28), whereas it has been shown that it inhibits cell proliferation in human colon carcinoma (29), melanoma cell lines (30-31), and M1 leukemia cells (32). In human breast carcinoma, IL-6 has been shown to have contradictory effects on cell proliferation. Badache and Hynes reported that IL-6 inhibited the proliferation of T47D breast cancer cells through STAT3 activation (23). Danforth and Sgagias also showed that IL-1 alpha and IL-6 additively inhibited the growth of MCF-7 breast cancer cells (33). On the other hand, Conze *et al*.

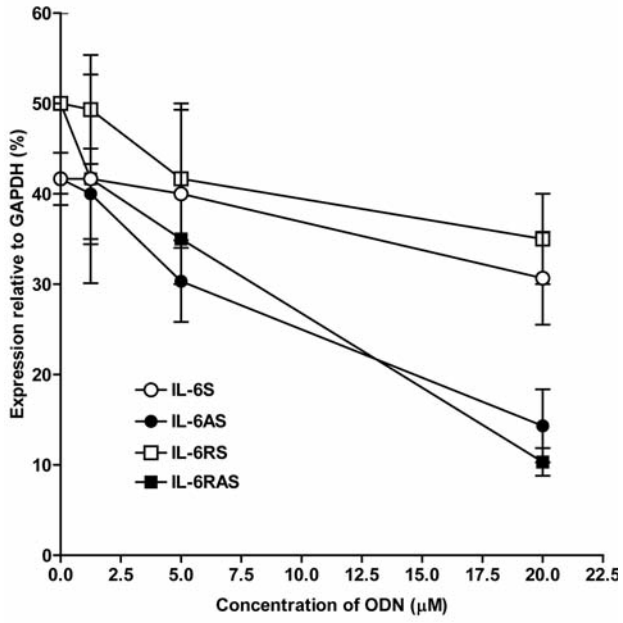

Figure 4. *Inhibition of IL-6 and IL-6R gene expression in MCF-7 cells by antisense IL-6 and IL-6R ODNs, respectively.*

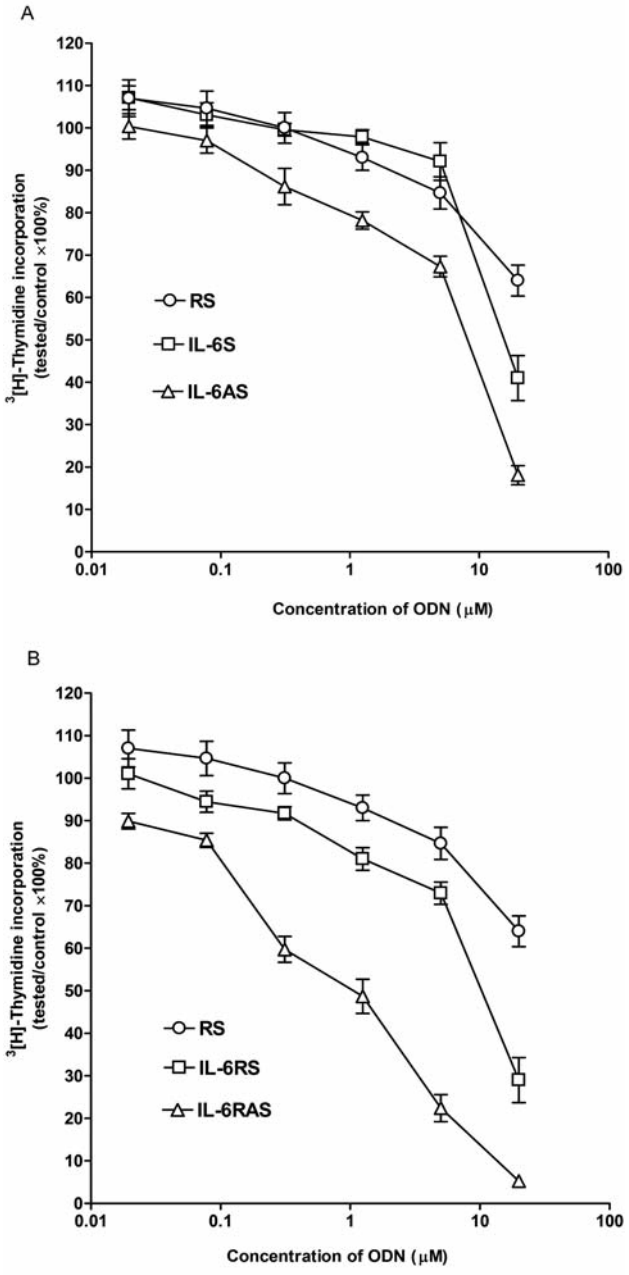

Figure 5. *IL-6 (A) and IL-6R (B) antisense ODN inhibited the growth of the MCF-7 breast cancer cells in a dose-dependent manner (72-hour incubation).*

reported that IL-6 induced mutidrug resistance in MCF-7 cells and had no significant effect on cell proliferation (34). Honma's group also found that IL-6 alone did not significantly effect cell proliferation of MCF-7 cells, but significantly increased the estrone sulfate-induced proliferation when IL-6 and estrone sulfate were simultaneously added to the culture media. They hypothesized that IL-6 regulated proliferation of breast cancer cells through estrogen production by steroid-catalyzing enzymes in the tissue (35). In agreement with Conze *et al*.'s and Honma *et al*.'s results, we did not find that the addition of human recombinant IL-6 and sIL-6R significantly stimulated or inhibited cell proliferation in MCF-7 breast cancer cells. The inhibition of proliferation by IL-6 in T47D, A375 melanoma and M1 leukemia cells was associated with STAT3 activation (23, 31-32), whereas the proliferation-promoting effect of IL-6 for some cell types was shown to involve SHP-2 and MAPK activation (32, 36). Thus, in some cells, the biological effect of IL-6 depends on the balance between a growth-inhibitory STAT3-dependent pathway and a growth-promoting MAPK/PI3K-dependent pathway (37-38). However, such a simplistic model for IL-6 action may not apply to all cell systems. For example, STAT3 itself can promote cell growth (39-40) and even behave as an oncogene when constitutively active (41). Thus, the molecular mechanism of IL-6 action on MCF-7 cells remains unclear.

We used IL-6 antibody to neutralize the IL-6 synthesized by MCF-7 cells and found that the antibody inhibited cell growth. This growth-inhibitory effect can be reversed by the addition of exogenous IL-6 to the culture media (Figure 2). These results suggested that IL-6 is required to maintain MCF-7 cell survival and growth. However, in the present study, the IL-6RAb had no inhibitory effect on MCF-7 cell growth. We increased the concentration of IL-6RAb in the culture medium, but high concentrations of IL-6RAb (>300 μg/ml) only led to non-specific inhibition of proliferation (Figure 2). It has previously been reported that monoclonal

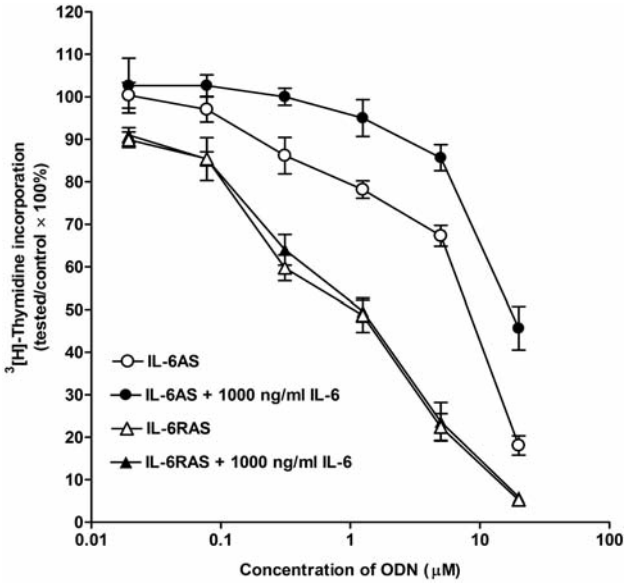

Figure 6. *The addition of exogenous human recombinant IL-6 partially abrogated the inhibitory effects of IL-6AS but not IL-6RAS ODNs in MCF-7 cells (72-hour incubation).*

Table I. *The effects of IL-6 and IL-6R sense and anti-sense ODNs on the secretion of IL-6 and sIL-6R proteins of MCF-7 cells. IL-6 and sIL-6R protein in culture media were measured by ELISA.*

	IL-6 (pg/10^6 cells)	*sIL-6R* (pg/10^6 cells)	Probability[a]
Control	6.1±1.8	164.4±15.7	
IL-6S (20 μM)	7.2±3.5	NT	NS
IL-6AS (20 μM)	3.0±0.5	NT	<0.05
IL-6RS (20 μM)	NT	159.2±20.1	NS
IL-6RAS (20 μM)	NT	70±11.2	<0.01

[a]Paired *t*-test, compared to control without any agent, n=3. Values are the mean±standard deviation. NT: Not tested; NS: not significant.

Table II. *IC_{50}s of random sequence, IL-6S, IL-6AS, IL-6RS and IL-6RAS ODNs in MCF-7 cells.*

	IC_{50} (μM)	Probability[a]
Random sequence	40.0±4.5	
IL-6S	18.0±5.1	<0.05[b]
IL-6AS	5.0±1.8	<0.01[c]
IL-6RS	15.0±3.9	<0.01[b]
IL-6RAS	1.0±0.35	<0.01[c]

[a]Paired *t*-test, n=3. Values are the mean±standard deviation. [b]Compared to antisense ODNs; [c]compared to random sequence.

antibodies directed to different regions of a protein may have different effects on cell proliferation (42). Therefore, multiple clones of monoclonal antibodies, directed to different epitopes of the IL-6R, need to be tested in MCF-7 human breast cancer cells to determine if the binding of IL-6 to the IL-6R can be disrupted.

To demonstrate the relationship between expression of *IL-6* and *IL-6R* mRNA and MCF-7 cell growth, we used IL-6 and IL-6R anti-sense ODNs to target *IL-6* and *IL-6R* genes in MCF-7 cells, respectively. mRNA levels were measured by RT-PCR and cell growth was evaluated by ^{3}[H]-thymidine incorporation. RT-PCR showed that mRNA levels of *IL-6* and the *IL-6R* in MCF-7 cells were reduced by IL-6 and IL-6R anti-sense ODNs, respectively, in a dose-dependent manner (Figure 4). The secretion of IL-6 and IL-6R protein was also suppressed by the corresponding anti-sense ODNs (Table I). ^{3}[H]-Thymidine incorporation showed that the proliferation of MCF-7 cells was also inhibited by IL-6 and IL-6R anti-sense ODNs in a dose-dependent manner (Figures 5 and 6). The cytotoxicity of IL-6R anti-sense ODN was five times greater than that for IL-6 anti-sense ODN (Table II). Furthermore, addition of IL-6 to the culture media partially abrogated the inhibitory effect on MCF-7 cells of IL-6 antisense ODN but not IL-6R antisense ODN (Figure 6). In agreement with Keller and Ershler's study (22), these results demonstrate that decreases in mRNA and protein expression of IL-6 and IL-6R are associated with the inhibition of cell growth, and that IL-6 and IL-6R anti-sense ODNs might be candidates for antisense cancer therapies.

Breast cancer patients have often been found to have increased serum IL-6 levels (16, 43-46). This IL-6, produced by the tumor cells, can neutralize the inhibitory effect of IL-6 anti-sense ODN on breast cancer cells. However, IL-6R anti-sense ODN can inhibit breast cancer cells in the presence of exogenous IL-6, and additionally, IL-6R anti-sense ODN are more cytotoxic than are IL-6 anti-sense ODN. Therefore, IL-6R anti-sense ODN might have greater efficacy in cancer therapy than IL-6 anti-sense ODN.

Cancer therapy through suppression of *IL-6* gene expression in cancer cells by curcumin has also been reported (47-50). Curcumin is a dihydroxyphenolic compound isolated from the rhizome of the plant *Curcuma longa*, and has anticancer, antiviral, antioxidant and anti-inflammatory properties. The underlying mechanisms of these effects are diverse and appear to involve the regulation of various molecular targets, including transcription factors (such as nuclear factor-κB), growth factors (such as vascular endothelial growth factor), inflammatory cytokines (such as tumor necrosis factor, IL-1 and IL-6) and protein kinases (such as MAPKs and AKT) (47). Saydmohammed *et al.* have reported that treatment of cancer cells with curcumin induces a dose- and time-dependent decrease in IL-6 expression and IL-6-induced phosphorylation of STAT3, which is associated with reduced cell viability and increased cleavage of caspase-3 (48). Park

et al. have also reported that treatment with curcumin dramatically inhibited the production of pro-inflammatory cytokines and vascular endothelial growth factor of human multiple myeloma U266 cells, and reduced IL-6/sIL-6R-induced STAT3 and ERK phosphorylation. In addition the combination of curcumin and bortezomib synergistically inhibited the growth of multiple myeloma (49).

In summary, the data in the present study demonstrates that IL-6 is a growth factor which maintains MCF-7 cell survival and growth. Neutralization of IL-6 with an IL-6-specific antibody or down-regulation of *IL-6* mRNA and protein with IL-6 antisense ODN resulted in growth inhibition of MCF-7 cells. Exogenous IL-6 can abrogate the inhibitory effects of both antibody and antisense ODNs. IL-6R antisense ODN reduced the expression of IL-6R mRNA and protein of MCF-7 cells, and suppressed MCF-7 cell growth. The cytotoxicity of IL-6R antisense ODN was greater than that for IL-6 antisense ODN and was not affected by addition of exogenous IL-6. In conclusion, IL-6 plays an important role in the growth of MCF-7 breast cancer cells, and careful modulation of IL-6 and IL-6R expression of cells by using antibodies and antisense ODNs, especially IL-6R anti-sense ODN, is a potential approach for breast cancer therapy.

Acknowledgements

This study was funded by The Sallie Astor Burdine Breast Foundation, Baton Rouge, Louisiana and The Delta State University Foundation, Cleveland, Mississippi, USA.

References

1 Kishimoto T: The biology of interleukin-6. Blood *14*: 1-10, 1990.

2 Kyle RA: Prognostic factors in multiple myeloma. Stem Cell (Dayt) *13(Suppl 2)*: 56-63, 1995.

3 Seymour JF, Talpaz M, Hagemeister FB, Cabanillas F and Kurzrock R: Clinical correlates of elevated serum levels of interleukin-6 in patients with untreated Hodgkin's disease. Am J Med *102*: 21-28, 1997.

4 Blay JY, Negrier S, Combaret V, Attali S, Goillot E, Merrouche Y, Mercatello A, Ravault A, Tourani JM and Moskovtckenko JF: Serum level of interleukin-6 as a prognosis factor in metastatic renal cell carcinoma. Cancer Res *52*: 3317-3322, 1992.

5 Yoshida N, Ikemoto S, Narita K, Sugimura K, Wada S, Yasumoto R, Kishimoto T and Nakatani T: Interleukin-6, tumour necrosis factor alpha and interleukin-1beta in patients with renal cell carcinoma. Br J Cancer *86*: 1396-1400, 2002.

6 Thiounn N, Pages F, Flam T, Tartour E, Mosseri V, Zerbib M, Beuzeboc P, Deneux L, Fridman WH and Debre B: IL-6 is a survival prognostic factor in renal cell carcinoma. Immunol Lett *58*: 121-124, 1997.

7 Andrews B, Shariat SF, Kim JH, Wheeler TM, Slawin KM and Lerner SP: Preoperative plasma levels of interleukin-6 and its soluble receptor predict disease recurrence and survival of patients with bladder cancer. J Urol *167*: 1475-1481, 2002.

8 Scambia G, Testa U, Benedetti PP, Foti E, Martucci R, Gadducci A, Perillo A, Facchini V, Peschle C and Mancuso S: Prognostic significance of interleukin-6 serum levels in patients with ovarian cancer. Br J Cancer *71*: 354-356, 1995.

9 Tempfer C, Zeisler H, Sliutz G, Haeusler G, Hanzel E and Kainz C: Serum evaluation of interleukin-6 in ovarian cancer patients. Gynecol Oncol *66*: 27-30, 1997.

10 Oka M, Yamamoto K, Takahashi M, Hakozaki M, Abe T, Iizuka N, Hazama S, Hirazawa K, Hayashi H, Tangoko A, Hirose K, Ishihara T and Suzuki T: Relationship between serum levels of interleukin-6, various disease parameters and malnutrition in patients with esophageal squamous cell carcinoma. Cancer Res *56*: 2276-2280, 1996.

11 Haverty AA, Harmey JH, Redmond HP and Bouchier-Hayes DJ: Interleukin-6 up-regulates GP96 expression in breast cancer. J Surg Res *69*: 145-149, 1997.

12 Yokoe T, Iino Y, Takei H, Horiguchi J, Koibuchi Y, Maemura M, Ohwada S and Morishito Y: Changes of cytokines and thyroid function in patients with recurrent breast cancer. Anticancer Res *17*: 695-699, 1997.

13 Tamm I, Cardinale I and Sehgal P: Interleukin-6 and 12-O-tetradecanoyl phorbol-13-acetate act synergistically in inducing cell-cell separation and migration of human breast carcinoma cells. Cytokine *3*: 212-223, 1991.

14 Hill RJ, Warren MK, Stenberg P, Levin H, Corash L, Drummond R, Bakers G, Levin F and Mok Y: Stimulation of megakaryocytopoiesis in mice by human recombinant interleukin-6. Blood *77*: 42-48, 1991.

15 Ullmann CD, Schlom J and Greiner JW: Interleukin-6 increases carcinoembryonic antigen and histocompatibility leukocyte antigen expression on the surface of human colorectal carcinoma cells. J Immunother *12*: 231-241, 1992.

16 Jiang XP, Yang DC, Elliott RL and Head JF: Interleukin-6 enhances tumor antigen expression in the MCF-7 human breast cancer cell line and breast cancer patients. Breast Cancer Res Treat *57*: 95a, 1999.

17 Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, Hirano T and Kishimoto T: Interleukin-6 triggers the association of its receptor with a possible signal transducer, GP130. Cell *58*: 573-581, 1989.

18 Kimura T, Wang J, Minamiguchi H, Fujiki H, Harada S, Okuda K, Kaneko H, Yokota S, Yasukawa K, Abe T and Sonoda Y: Signal through GP130 activated by soluble interleukin-6 receptor (IL-6R) and IL-6 or IL-6R/IL-6 fusion protein enhances ex vivo expansion of human peripheral blood-derived hematopoietic progenitors. Stem Cells *18*: 444-452, 2000.

19 Sui X, Tsuji K, Ebihara Y, Tanaka R, Muraoka K, Yoshid AM, Yamada K, Yasukawa K, Taga T, Kishimoto T and Nakahata T: Soluble interleukin-6 (IL-6) receptor with IL-6 stimulates megakaryopoiesis from human CD34(+) cells through glycoprotein (GP)130 signaling. Blood *93*: 2525-2532, 1999.

20 Yasukawa K, Hirano T, Watanabe Y, Muratani K, Matsuda T, Nakai S and Kishimoto T: Structure and expression of human cell stimulatory factor-2 (*BSF-2/IL-6*) gene. EMBO J *6*: 2939-2949, 1987.

21 Schooltink H, Stoyan T, Lenz D, Schmitz H, Hirano T, Kishimoto T, Henrich P and Rose-John S: Structural and functional studies on the human hepatic interleukin-6 receptor. Molecular cloning and overexpression in HepG2 cells. Biochem J *277*: 659-664, 1991.

22 Keller T and Ershler WB: Effect of IL-6 receptor antisense oligodeoxynucleotide on *in vitro* proliferation of myeloma cells. J Immunol *154*: 4091-4098, 1995.
23 Badache A and Hynes NE: Interleukin-6 inhibits proliferation and, in cooperation with an epidermal growth factor receptor autocrine loop, increases migration of T47D breast cancer cells. Cancer Res *61*: 383-391, 2001.
24 Steiner H, Godoy-Tundidor S, Rogatsch H, Berger AP, Fuchs D, Comuzzi B, Bartsch G, Hobisch A and Culig Z: Accelerated *in vivo* growth of prostate tumors that up-regulate interleukin-6 is associated with reduced retinoblastoma protein expression and activation of the mitogen-activated protein kinase pathway. Am J Pathol *162*: 655-663,2003.
25 Schneider MR, Hoeflich A, Fischer JR, Wolf E, Sordat B and Lahm H: Interleukin-6 stimulates clonogenic growth of primary and metastatic human colon carcinoma cells. Cancer Lett 151, 31-38, 2000.
26 Reittie JE, Yong KL, Panayiotidis P and Hoffbrand AV: Interleukin-6 inhibits apoptosis and tumour necrosis factor induced proliferation of B-chronic lymphocytic leukaemia. Leuk Lymphoma 22: 83-90, 1996.
27 Lu C and Kerbel RS: Interleukin-6 undergoes transition from paracrine growth inhibitor to autocrine stimulator during human melanoma progression. J Cell Biol *120*: 1281-1288, 1993.
28 Koo AS, Armstrong C, Bochner B, Shimabukuro T, Tso C, Dekernion JB and Belldegrun A: Interleukin-6 and renal cell carcinoma: production, regulation, and growth effects. Cancer Immunol. Immunother *35*: 97-105, 1992.
29 Xiao B, Jing B, Zhang YL, Zhou DY and Zhang WD: Tumor growth inhibition effect of hIL-6 on colon cancer cells transfected with the target gene by retroviral vector. World J Gastroenterol *6*: 89-92, 2000.
30 Fontaine V, Mahieu M and Content J: Interferon-gamma and interleukin-6 inhibit proliferation in human melanoma cells by different signalling pathways. Melanoma Res *8*: 24-30, 1998.
31 Kortylewski M, Heinrich PC, Mackiewicz A, Schniertshauer U, Klingmuller U, Nakajima K, Hirano T, Horn F and Behrmann I: Interleukin-6 and oncostatin M-induced growth inhibition of human A375 melanoma cells in STAT-dependent and involves up-regulation of the cyclin-dependent kinase inhibitor p27/KIP1. Oncogene *18*: 3742-3753, 1999.
32 Nakajima K, Yamanaka Y, Nakae K, Kojima H, Ichiba M, Kiuchi N, Kitaoka T, Fukada T, Hini M and Hirano T: A central role for STAT3 in IL-6-induced regulation of growth and differentiation in M1 leukemia cells. EMBO J *15*: 3651-3658, 1996.
33 Danforth DN Jr., and Sgagias MK: Interleukin-1 alpha and interleukin-6 act additively to inhibit growth of MCF-7 breast cancer cells *in vitro*. Cancer Res *53*: 1538-1545, 1993.
34 Conze D, Weiss L, Regan PS, Bhushan A, Weaver D, Johnson P and Rincon M: Autocrine production of interleukin-6 causes multidrug resistance in breast cancer cells. Cancer Res *61*: 8851-8858, 2001.
35 Honma S, Shimodaira K, Shimizu Y, Tsuchiya N, Saito H, Yanaihara T and Okai T: The influence of inflammatory cytokines on estrogen production and cell proliferation in human breast cancer cells. Endocr J *49*: 371-377, 2002.
36 Fukada T, Hibi M, Yamanaka Y, Takahishi-Tezuka M, Fujitani Y, Yamaguchi T, Nakajima K and Hirano T: Two signals are necessary for cell proliferation induced by a cytokine receptor GP130: involvement of STAT3 in anti-apoptosis. Immunity *5*: 449-460, 1996.
37 Hirano T: Molecular basis underlying functional pleiotropy of cytokines and growth factors. Biochem Biophys Res Commun *260*: 303-308, 1999.
38 Leu CM, Wong FH, Chang C, Huang SF and Hu CP: Interleukin-6 acts as an antiapoptotic factor in human esophageal carcinoma cells through the activation of both STAT3 and mitogen-activated protein kinase pathways. Oncogene *22*: 7809-7818, 2003.
39 Fukada T, Ohtani T, Yoshida Y, Shirogane T, Nishida K, Nakajima K, Hibi M and Hirano T: STAT3 orchestrates contradictory signals in cytokine-induced G1 to S cell-cycle transition. EMBO J *17*: 6670-6677, 1998.
40 Horiguchi A, Oya M, Marumo K and Murai M: STAT3, but not ERKs, mediates the IL-induced proliferation of renal cancer cells, ACHN and 769P. Kidney Int *61*: 926-938, 2002.
41 Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C and Darnell JE: *STAT3* as an oncogene. Cell *98*: 295-303, 1999.
42 Corada M, Liao F, Lindgren M, Lampugnani MG, Breviario F, Frank R, Muller WA, Hicklin DJ, Bohlen P and Dejana E: Monoclonal antibodies directed to different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability. Blood *97*: 1679-1684, 2001.
43 Jiang, XP, Yang DC, Elliott RL and Head JF: Reduction in serum IL-6 after vaccination of breast cancer patients with tumor-associated antigens is related to estrogen receptor status. Cytokine *12*: 458-465, 2000.
44 Jiang XP, Yang DC, Head JF and Elliott RL: All-*trans*-retinoic acid down-regulates interleukin-6 synthesis in human breast cancer. Proc Am Assoc Cancer Res *40*: 63, 1999.
45 Jiang XP, Yang DC, Head JF and Elliott RL: Vaccination of breast cancer patients with autologous tumor-associated antigens results in reduction of serum interleukin-6. Proc Am Assoc Cancer Res *39*: 356, 1998.
46 Head JF, Jiang XP, Yang DC and Elliott RL: Serum interleukin-6 (IL-6) concentration correlates with serum tumor marker CA15-3 concentration in breast cancer patients. Proc Am Soc Clin Oncol *18*: 630a, 1999.
47 Zhou H, Beevers CS and Huang S: The targets of curcumin. Curr Drug Targets *12*: 332-347, 2011.
48 Saydmohammed M, Joseph D and Syed V: Curcumin suppresses constitutive activation of STAT-3 by up-regulating protein inhibitor of activated STAT-3 (*PIAS-3*) in ovarian and endometrial cancer cells. J Cell Biochem *110*: 447-456, 2010.
49 Park J, Ayyappan V, Bae EK, Lee C, Kim BS, Kim BK, Lee YY, Ahn KS and Yoon SS: Curcumin in combination with bortezomib synergistically induced apoptosis in human multiple myeloma U266 cells. Mol Oncol *2*: 317-326, 2008.
50 Cohen AN, Veena MS, Srivatsan BS and Wang MB: Suppress of interleukin-6 and -8 production in head and neck cancer cells with curcumin *via* inhibition of Ikappa beta kinase. Arch Otolaryngol Head Neck Surg *135*: 190-197, 2009.

Received June 5, 2011
Revised July 4, 2011
Accepted July 5, 2011