Influence of Interleukin-6 on the Invasiveness of Human Colorectal Carcinoma

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Abstract. *Background: Plasma interleukin-6 (IL-6) level correlates with patient survival in colorectal carcinoma and is regarded as a prognostic factor. In this study, the role of IL-6 in colorectal carcinoma proliferation, chemotaxis and invasion was investigated. Materials and Methods: Proliferation and invasion were measured in four colorectal carcinoma cell lines. The effect of IL-6 (10, 50, 100 ng/ml) on progression (the ability to grow, adhere, chemotax and invade) was also measured. Results: Physiological (10 ng/ml) and pharmacological (50 and 100 ng/ml) concentrations of IL-6 did not significantly affect growth. However, IL-6 (10 ng/ml) significantly increased attachment to basement membrane ($p<0.05$). IL-6 (50 ng/ml) significantly increased the chemotaxis, anchorage-independent growth and invasiveness of SW-480 (one of the four cell lines) ($p<0.05$). IL-6 (100 ng/ml) resulted in negative feedback inhibition of these effects. Conclusion: IL-6 plays an important role in the progression of colorectal carcinoma.*

Interleukin-6 (IL-6) is a pleiotropic cytokine that affects hematopoiesis, the immune response and the acute-phase response. Originally, IL-6 was identified as a T-cell-derived factor that triggers antibody secretion and B cell maturation (1). However, molecular cloning demonstrated that IL-6 is a highly multifunctional protein that participates in many physiological and pathological responses to disease, including inflammation, myocardial infarction, autoimmune disorders and hematological malignancies (2-5). Interest in the effects of IL-6 on solid tumors has grown because many carcinomas produce cytokines, which can act as autocrine growth factors (6-8). The IL-6 and interleukin-6 receptor (IL-6R) autocrine loop have been identified in esophageal carcinoma, renal cell carcinoma and multiple myeloma (9-11). Although serum IL-6 level was reported to increase and correlate with disease status and prognosis in patients with various malignant diseases (7, 12-16), how IL-6 affects carcinoma cells is still under investigation.

IL-6 binds to a transmembrane receptor, gp80 (IL-6R, α chain), in cancer cells and subsequently associates with the signal transducing β chain of the IL-6R complex, gp 130 (17). The resulting signal activates a cytoplasmic tyrosine kinase that triggers the Janus-activated kinase (JAK)/signal transducers and then activates transcription (STAT) proteins, the Ras/mitogen-activated protein kinase (MAPK) pathway, and phosphatidylinositol 3-kinase (PI3K) /AKT signaling (18, 19). IL-6 signaling regulates many cellular functions, such as cell growth and survival, differentiation, cell motility and angiogenesis (20-22). Adding IL-6 has been reported to increase proliferation of colorectal carcinoma cells (23, 24). Schneider *et al.* have also reported that IL-6 could stimulate clonogenic growth of colon carcinoma cells (25). Other reports have indicated that IL-6 stimulated the expression of surface antigens, such as CEA and HLA class I molecule (26, 27). None of these investigations have assessed the effect of IL-6 on the invasiveness of cancer cells. The present study was designed to evaluate whether IL-6 could enhance colorectal carcinoma cell invasiveness and to elucidate the possible role of IL-6 in the progression of colorectal carcinoma.

Materials and Methods

Cell lines and cell culture. The colon carcinoma cell lines, SW-480, LS1034, LOVO and HT-29 were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). Different culture media (Leibovitz [L-15], RPMI-1640, F-12 nutrient mixture and McCoy 5A) and fetal bovine serum (FBS) were from Gibco (Gibco Ltd, Paisley, UK). Cells within passage 5-30 were used for all experiments. For cytokine stimulation of proliferation, cells were incubated with IL-6 (0, 10, 50, and 100 ng/ml) or IL-6R (0, 0.1 and 1.0 ng/ml).

Proliferation assay. IL-6 or IL-6R was added or not to cells resuspended in culture media with 0.5% FBS which were distributed ($1x10^4$ cells/well) in 96-well flat-bottomed plates (final

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volume 200 μl). After 4 days at 37˚C and in a 5% CO_2 atmosphere (except SW480), proliferation was assessed by 3-[4, 5-dimethylthiazol-2yl]-2, 5-diphenyl-tetrazolium bromide (MTT)-conversion, as previously described (28).

Clonogenic growth in soft agar. Only SW480 was selected for the anchorage-independent study. Cells were plated in soft agar for clonogenicity essentially, as described by O'Brien *et al.* and Jeha *et al.* (29, 30). Briefly, a 60-mm culture dish was coated with a base feeder layer consisting of medium supplemented with 20% serum and 0.5% agarose. Then a top layer of $5x10^4$ cells with 20% serum and 0.35% agarose in 2 ml complete medium with IL-6 (0, 10, 50 and 100 ng/ml) was added. Culture was at 37˚C for 10 days. Colonies were stained with MTT (1 mg/ml) at 37˚C for 12 h and were then counted.

Adhesion assay. The 24-cell tissue culture plates were coated with 25 μg/well Matrigel and left to air dry in a hood overnight. To block nonspecific binding sites, all wells were incubated with culture medium containing 0.1% BSA for 1 h at 37˚C and were then washed with the same medium. SW480 cells ($1x10^5$ cells/ml) in medium were incubated with different concentrations of IL-6 at 37˚C for 1 h to allow restoration of the surface proteins. Aliquots (1 ml) of the tumor suspension were then seeded into the Matrigel-coated wells and incubated for 10 min, 30 min, or 60 min at 37˚C in 5% CO_2. At the end of these periods, the wells were washed gently three times with PBS to remove the unattached cells, whereas the attached cells were harvested and counted in 10 randomly selected microscopic fields (x400).

Invasion and chemotaxis assays. The invasion assay was carried out as described by Cos *et al.* (31) in modified Boyden's chambers constructed using multi-well cell culture plates and inserts (Falcon, Lincoln Park, NJ, USA). A layer of basement membrane was applied to the surfaces of microporous filters in each unit. The upper chamber was coated with Matrigel (25 μg per filter), which served as the invasion barrier, whereas fibronectin (16 μg per filter, which acted as a chemoattractant, was placed in the lower chamber. SW 480 cells ($5x10^4$ cells per chamber) were added to the upper chamber with different concentrations of IL-6 in culture medium and were incubated at 37˚C for 5 days. The cells that invaded the basement membrane into the lower chamber were fixed, stained with hematoxylin and eosin and counted in 10 randomly selected microscopic fields (x400).

The chemotaxis assay was performed as described for the invasion assay except that the filter in the upper chamber was coated with collagen IV (5 μg per filter; Becton Dickson) instead of Matrigel. This coats the interstices of the filter but does not form a barrier over the surface. All experiments were performed four times in triplicate.

Statistical analysis. The data were analyzed by a one-way ANOVA followed by the Student-Newman-Keuls multiple comparisons test. Differences among the groups' means were considered significant at $p<0.05$.

Results

Proliferation assay. In all four lines, IL-6 (10, 50, 100 ng/ml) failed to significantly increase cell numbers after 48-h incubation. The increase in LS1034, LOVO and HT-29 cells was not consistent. The number of SW480 cells increased when IL-6 (<50 ng/ml) was present for less than 24 h but decreased when IL-6 (<50 ng/ml) was present for more than 24 h (Figure 1). IL-6R (at all concentrations) failed to stimulate proliferation of all four cell lines. In addition, proliferation was inconsistent in all cell lines during the 48-h observation period (Figure 2).

Anchorage-independent clonogenic growth. SW-480 was chosen for further studying because its cell numbers partially correlated with IL-6 concentration. Exogenous IL-6 (10 ng/ml and 50 ng/ml) significantly increased, SW-480 colony numbers after culture for 10 days ($p<0.05$). However, IL-6 (100 ng/ml) retarded growth (Figure 3).

Adhesion. To evaluate the influence of IL-6 on the attachment of SW480 cells to the basement membrane, cells were added to tissue culture plates coated with basement membrane components (Matrigel) and were incubated for 10 min, 30 min, or 60 min in the presence of different concentrations of IL-6. IL-6 (10 ng/ml) influenced the adhesiveness of cells (the number of cells adhering to the wells) at 30 min and 60 min but not at 10 min ($p<0.05$). IL-6 (50 ng/ml and 100 ng/ml), on the contrary, reduced adhesiveness (Figure 4).

Invasion and chemotaxis ability. When IL-6 (50 ng/ml and 100 ng/ml, but not 10 ng/ml) were added to the upper compartment of the invasion chamber, the number of cells that invaded the Matrigel membrane increased significantly ($p<0.05$) compared with that of the control group. The invasion was most prominent at 50 ng/ml of IL-6 (Figure 5). The introduction of fibronectin into the lower compartment accelerated the movement of the cells from upper compartment through the collagen IV membrane separating the upper from the lower chambers. IL-6 (50 ng/ml but not other concentrations) significantly increased this migration ($p<0.05$) (Figure 5). The statistical analysis of these data is summarized in Table I.

Discussion

Metastasis proceeds *via* several sequentially linked steps, including tumor growth, establishment of a blood supply at the primary site, invasion, release into blood vessels or lymphatics, arrest at a distant site, extravasations and proliferation as a secondary colony with angiogenesis. Each step is strictly regulated by complex interactions occurring between tumors and hosts. The mechanisms of metastasis are now thought to be similar to those of inflammatory processes because many of the same cytokines are involved in both reactions. In the present study, using the cell line SW 480, exogenous IL-6 was shown to result in progression

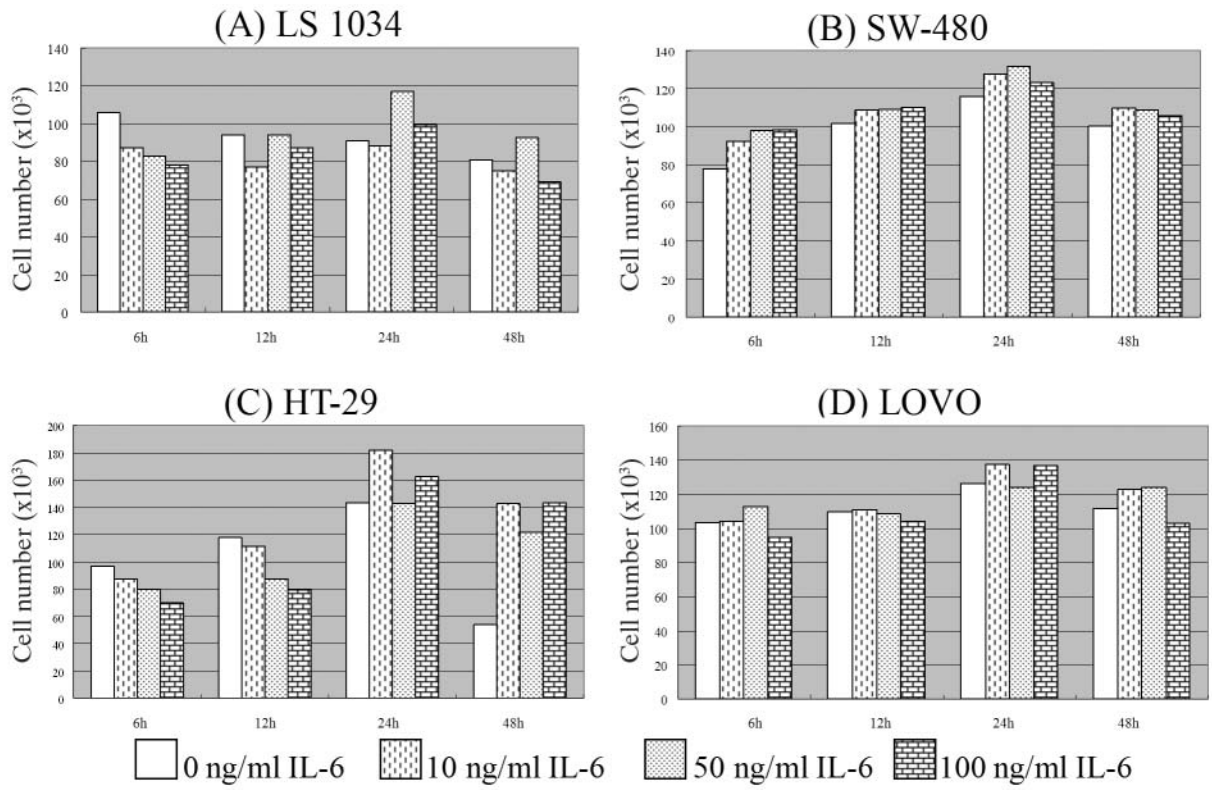

Figure 1. *Four cell lines (LS 1034, SW 480, HT-29 and LOVO) were stimulated with IL-6 (10, 50, 100 ng/ml) and cultured for 48 h. Cell proliferation was measured by the MTT method. SW 480 cell numbers increased when the concentration was below 50 ng/ml and the culture period was under 24 h. The growth of other cell lines was inconsistent during the 48-h culture period.*

Table I. *Summary of the changes of biological properties of SW 480 after exogenous IL-6 was supplied.*

Biological property	Control	10 ng/ml	50 ng/ml	100 ng/ml
Cellular proliferation (MTT method, 24 h)	115.82	127.57	131.91	123.30
Clonogenic growth in soft agar	6.5±1.0	12±0.5	17±1.0	5.5±1.0
Adhesion effect (% of control)				
30 min	100	126.0±10.4*	94.9±4.3	79.2±4.9
60 min	100	130.4±12.5*	94.5±5.4	86.1±7.2
Chemotaxis ability (% of control)	100	123.5±10.4	147.7±12.3*	129.9±7.5
Invasive ability (% of control)	100	57.0±12.0	826.0±14.3*	308.1±29.2*

*when compared to control group, $p<0.05$.

of metastasis through enhancement of multiple events in this cascade, such as anchorage-independent clonogenic growth, adhesion, chemotaxis and invasion.

However, the growth promoting activity of IL-6 in colorectal cancer cell lines was inconsistent. IL-6-stimulated proliferation was manifested only as a trend and only in SW 480. This result is consistent with previous reports (24, 32) showing that IL-6 stimulates proliferation of only selected colorectal cell lines. Our findings can be explained by assuming that SW 480 expresses IL-6R mRNA but not IL-6 mRNA. Therefore, interaction of exogenous IL-6 with IL-6R may be an important signaling mechanism. However, the expression of IL-6 mRNA and IL-6R mRNA in the other cell lines is different from that in SW480. LS 1034 expresses neither IL-6 mRNA nor IL-6R mRNA, LOVO expresses both and HT-29 expresses IL-6 mRNA but not IL-6R mRNA (25).

In this study, cell lines possessing different IL-6 and IL-6R expression capabilities had different proliferative capabilities. Previous reports suggested a promitogenic/comitogenic role for IL-6/IL-6R/STAT3 signaling in cell proliferation and a potential role for IL-6/IL-6R/STAT3 in the growth and progression of numerous

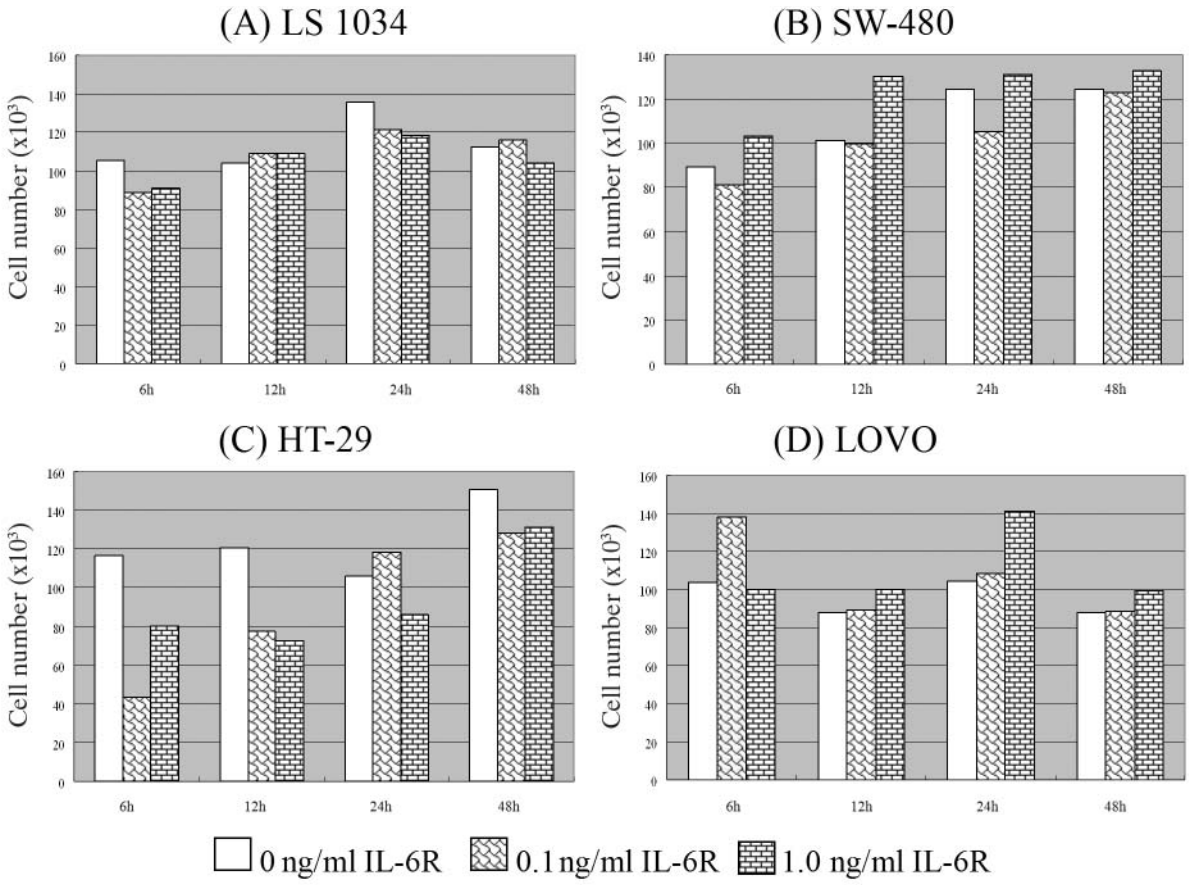

Figure 2. *Four cell lines (LS 1034, SW 480, HT-29 and LOVO) were cultured in the presence of IL-6R (0.1, 1.0 ng/ml) for 48 h. Cell numbers were counted using the MTT method. The growth of the four cell lines was not consistent during the 48-h incubation period.*

tumor types (33). However, IL-6 was also reported to inhibit the growth of hepatocellular carcinoma (34), prostate cancer (35) and osteosarcoma cells (36). Thus, a better understanding of the intracellular signaling mechanism leading to either growth stimulation or inhibition by IL-6 is needed. In this study, the proliferation of SW 480 was measured by the MTT assay and colony formation. The two systems, however, measure different parameters. The methylcellulose assay reflects the number of cells that originally divided to give rise to a colony. Since clonogenic growth in soft agar correlates best with *in vivo* tumorigenicity (30, 37), both methods were used to assess proliferation of SW 480. Indeed, the MTT assay showed only an increasing trend after IL-6 addition, while the colony formation method showed a significant difference. Thus, the results of the clonogenic assay suggest that IL-6 might be a stimulator of primary and metastatic colorectal carcinoma growth *in vivo*.

Recently, we have demonstrated that serum IL-6 level was associated with the advanced staging and poor prognosis of patients with colorectal carcinoma (15). However, the actual role of IL-6 in the progression of colorectal carcinoma was rather complex. Motility and attachment are two key cellular functions for the process of metastasis. The enhancement of one or more of these cellular functions may increase the metastatic potential of the tumor. This study confirmed that exogenous IL-6 could enhance adhesion, chemotaxis and invasiveness of SW 480. Tumor cell motility and invasion are adhesion-dependent phenomena related to the presence of cell surface adhesion molecules for both cell-cell and cell-matrix interaction (38-40). Chen *et al.* reported thet IL-6 induced MMP-3 and MMP-9 expression and activation in mouse brain and increased proliferation and migration of cerebral endothelial cells. Furthermore, they confirmed that IL-6 mRNA expression strongly correlated with the expressions of MMP-3 and MMP-9 mRNA (41). Another report also suggested that IL-6 triggered angiogenesis through stimulation of MMP-9 and vascular endothelial growth factor (VEGF) overexpression (42). IL-6 also mediated the up-regulation of cardiac ICAM activity following trauma hemorrhage and induced ICAM gene

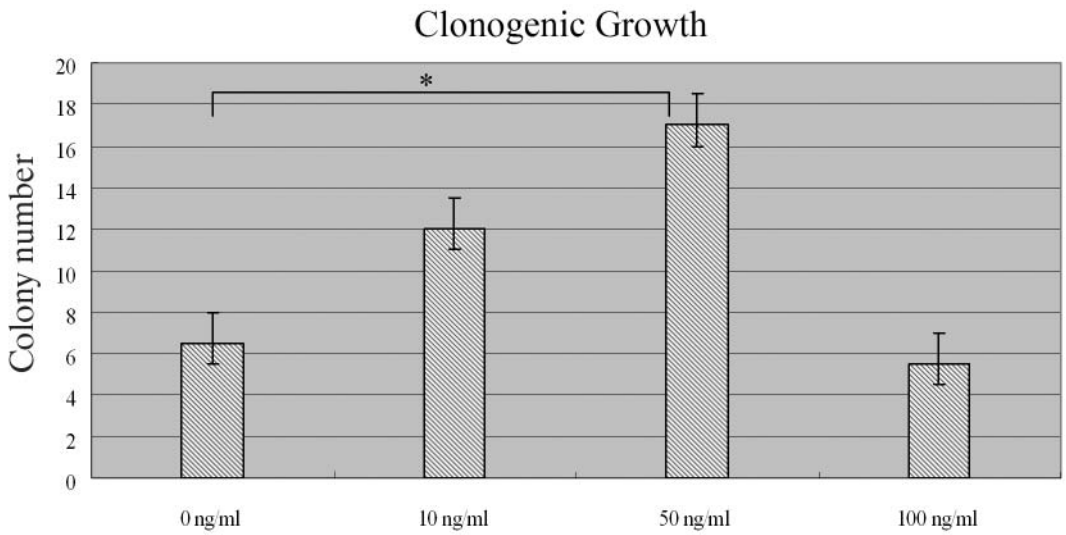

Figure 3. *The effect of IL-6 on the clonigenic growth of SW 480 was examined. The growth was significantly enhanced by IL-6 (50 ng/ml) (*p<0.05 compared between 0 and 50 ng/ml groups), but inhibited by IL-6 (100 ng/ml).*

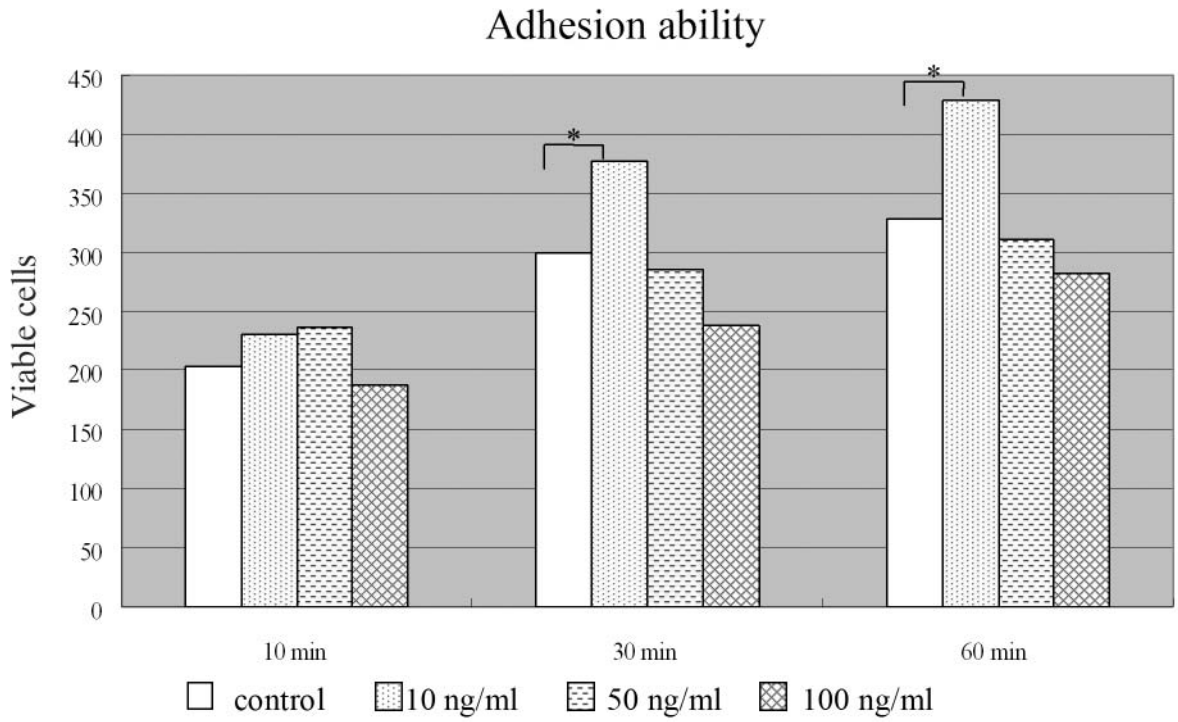

Figure 4. *The effect of exogenous IL-6 on cellular adhesion of SW 480. Adhesion was significantly increased by IL-6 (10 ng/ml) at 30 min and 60 min (*p<0.05 compared between 0 and 10 ng/ml groups), but not by IL-6 (50 and 100 ng/ml).*

expression (43, 44). Our result that IL-6 enhanced adhesion of SW 480 cells could be explained by the involvement of adhesion molecules in this process.

In our study, treatment of colorectal carcinoma cells with IL-6 significantly increased migration through the basement membrane, the number of adhering cells, chemotaxis and invasion. Blood supplies a tumor cell attached to the vessel endothelium with oxygen and nutrients. However, as a tumor invades, angiogenesis is needed for survival. The IL-6 and IL-6R autocrine loop in colorectal carcinoma provide a local

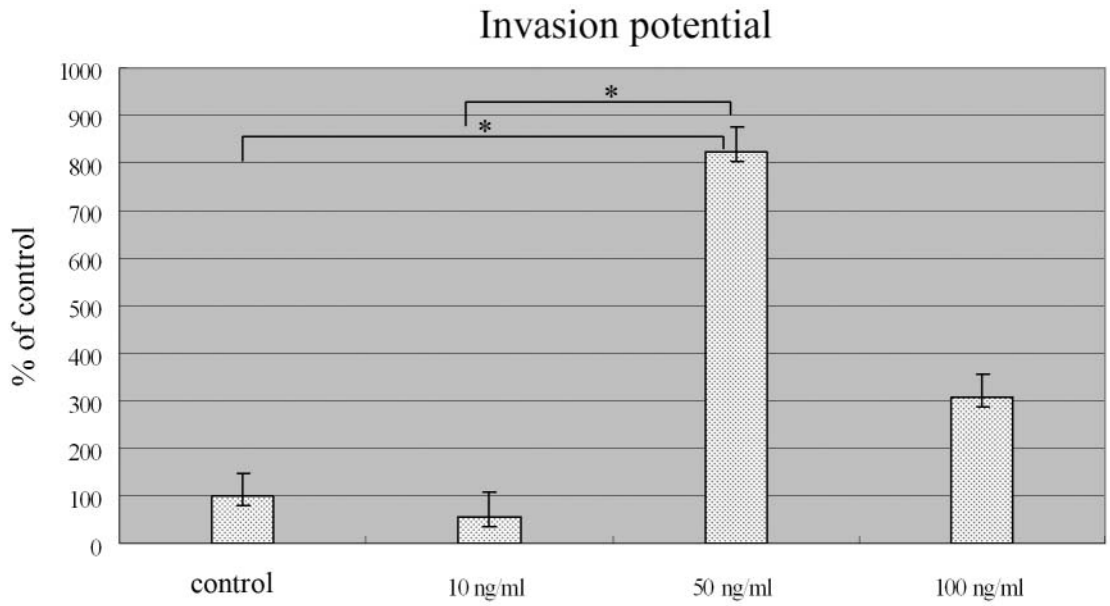

Figure 5. *The effect of exogenous IL-6 on SW 480 cell invasion. Invasiveness was increased significantly by IL-6 (50 ng/ml) (*p<0.05 compared with each group). At higher concentration (100 ng/ml), invasiveness decreased but remained significantly higher than that of control group (p<0.05).*

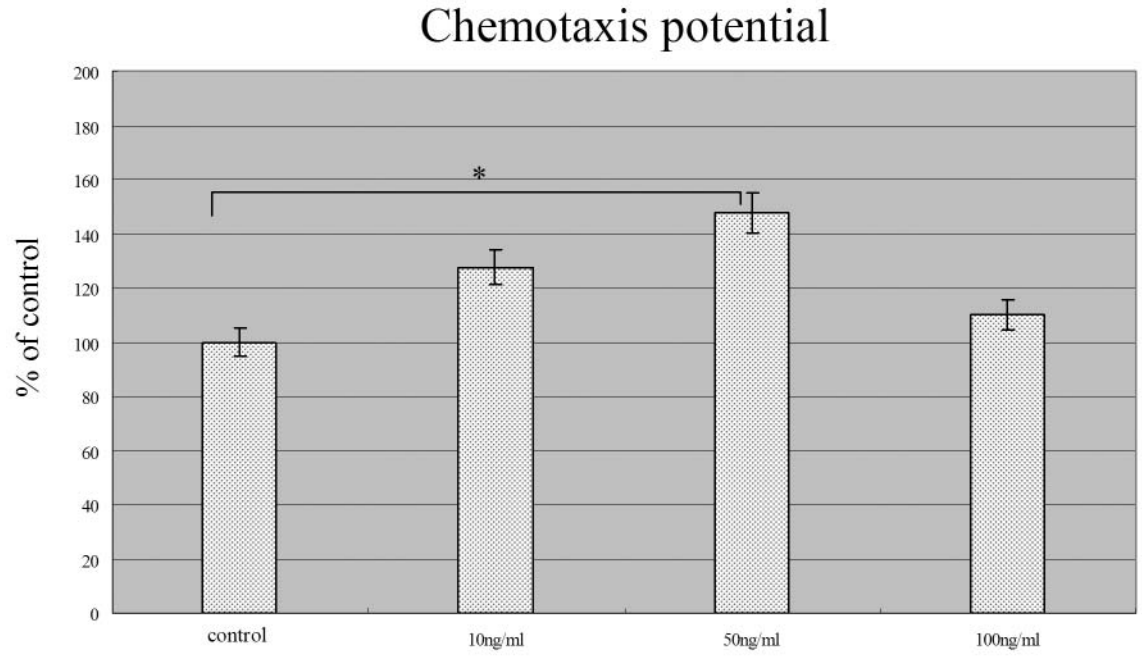

Figure 6. *The effect of exogenous IL-6 on SW 480 cell chemotaxis. Cell number was significantly increased in the presence of IL-6 (50 ng/ml) (*p<0.05 between the control and 50 ng/ml groups), but chemotaxis was inhibited by IL-6 (100 ng/ml).*

environment more favorable to tumor growth (16). Previously, the reports of Wei and Su *et al.* showed that IL-6 up-regulated VEGF *via* the STAT3 pathway to promote angiogenesis (45, 46). These findings are consistent with ours, showing that IL-6 stimulates chemotaxis and invasion of SW 480 cells.

Only therapeutic concentrations of IL-6 stimulated SW 480 clonogenic growth, adhesion, chemotaxis and invasion in our study. At higher concentrations, exogenous IL-6 might have an important role in controlling cellular progression under a negative feedback mechanism. The

role of IL-6 in intracellular signal transduction has been studied extensively, and its involvement in negative feedback regulation has been identified. Many reports have indicated that suppression of cytokine signaling (SOCS 3) protein is induced by various inflammatory cytokines, such as IL-6, IL-12, INF-γ, and IL-10, and it negatively regulates the activities of those cytokines, as well as STAT functions (47, 48). The report of Sommers *et al.* (49) suggested that SOCS 3 was a key negative regulator of IL-6. Their findings at least partially explain the association of IL-6 with the progression of colorectal carcinoma.

This study focused on the progression induced by IL-6 in colorectal carcinoma. Tumor invasion depends on the concentration of exogenous IL-6, which was found to induce significant changes in tumor behavior.

In conclusion, the current study disclosed that IL-6 affects the adhesion, chemotaxis and invasion of colorectal carcinoma, suggesting that IL-6 may be a useful clinical aid in the management of patients with this disease.

References

1 Hirano T, Yasukawa K, Harada H *et al*: Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature *324*: 73-76, 1986.

2 Ishihara K and Hirano T: Interleukin-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev *13*: 357-368, 2002.

3 Nian M, Lee P, Khaper N and Liu P: Inflammatory cytokines and postmyocardial infarction remodeling. Circ Res *94*: 1543-1553, 2004.

4 Kurzrock R: Cytokine deregulation in hematological malignancies: clinical and biological implications. Clino Cancer Res *3*: 2581-2584, 1997.

5 Trikha M, Corringham R, Klein B and Rossi JF: Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rational and clinical evidence. Clin Cancer Res *9*: 4653-4665, 2003.

6 Burger RA, Grosen EA, Ioli GR *et al*: Spontaneous release of interleukin-6 by primary culture of lymphoid and tumor cell populations purified from human ovarian carcinoma. J Interferon Cytokine Res *15*: 255-260, 1995.

7 Wu CW, Wang SR, Chao MF *et al*: Serum interleukin-6 levels reflect disease status of gastric cancer. Am J Gastroenterol *91*: 1417-1422, 1996.

8 Lu C, Sheehan C, Rak JW *et al*: Endogenous interleukin-6 can function as an *in vivo* growth stimulatory factor for advanced stage human melanoma cells. Clin Cancer Res *2*: 1417-1425, 1996.

9 Oka M, Iizuka N, Yamamoto K *et al*: The influence of interleukin-6 on the growth of human esophageal cancer cell lines. J Interferon Cytokine Res *16*: 1001-1006, 1996.

10 Kawano M, Hirano T, Matsuda T *et al*: Autocrine generation and requirement of BSF-2/ IL-6 for human multiple myeloma. Nature *332*: 83-85, 1988.

11 Miki S, Iwano M, Miki Y *et al*: Interleukin-6 (IL-6) functions as an *in vitro* autocrine growth factor in renal cell carcinoma. FEBS letter *250*: 607-610, 1989.

12 Riedel F, Zaiss I and Herzog D: Serum levels of interleukin-6 in patients with primary head and neck squamous cell carcinoma. Anticancer Res *25*: 2761-2766, 2005.

13 Salgado R, Junius S, Benoy I *et al*: Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. Int J Cancer *103*: 642-646, 2003.

14 George DJ, Halabi S, Shepard TF *et al*: The prognostic significance of plasma interleukin-6 levels in patients with metastatic hormone-refractory prostatic cancer: results from cancer and leukemic group B 9480 Clin Cancer Res *11*: 1815-1820, 2005.

15 Chung YC and Chang YF: Serum interleukin-6 levels reflect the disease status of colorectal cancer J Surg Oncol *83*: 222-226, 2003.

16 Kinoshita T, Ito H and Miki C: Serum interleukin-6 level reflects the tumor proliferate activity in patients with colorectal carcinoma. Cancer *85*: 2526-2531, 1999.

17 Kishimoto T, Akira S, Narazaki M, *et al*: Interleukin-6 family of cytokines and gp 130. Blood *86*: 1243-1254, 1995.

18 Taga T and Kishimoto T: GP130 and the interleukin-6 family of cytokines. Annu Rev Immuno *15*: 797-819, 1997.

19 Heinrich PC, Behrmann I, Haan S *et al*: Principles of interleukin-6 type cytokine signaling and its regulation. Biochem J *374*: 1-20, 2003.

20 Hirano T, Ishihara K and Hibi M: Roles of STAT3 in mediating the cell growth differentiation and survival signals related through the IL-6 family cytokine receptors. Oncogene *19*: 2548-2556, 2000.

21 Jee SH, Chu CY, Chiu HC *et al*: Interleukin-6 induced basic fibroblast growth factor dependent angiogenesis in basal cell carcinoma cell line *via* JAK/STAT3 and PI3 kinase/AKT pathway. J Invest Dermatol *123*: 1169-1175, 2004.

22 Leu CM, Wong FH, Chang C *et al*: Interleukin-6 acts as an antiapoptotic factor in human esophageal carcinoma cells through the activation of both STAT3 and mitogen-activated protein kinase pathway. Oncogene *22*: 7809-7818, 2003.

23 Köbel M, Budianto D, Schmitt WD *et al*: Influence of various cytokine on adhesion and migration of the colorectal adencarcinoma cell line HRT-18 Oncology *68*: 33-39, 2005.

24 Lahm H, Petral-Malec D, Yilmaz-Ceyhan A *et al*: Growth stimulation of human colorectal carcinoma cell line by interleukin-1 and antagonistic effects of transforming growth factor β1. Euro J Cancer *28*: 1894-1899, 1992.

25 Schneider MR, Hoeflich A, Fischer JR *et al*: Interleukin-6 stimulates clonogenic growth of primary and metastatic human colon carcinoma cells. Cancer Lett *151*: 31-38, 2000.

26 Tsang KY, Kashmiri SV, Qi CF *et al*: Transfer of the IL-6 gene into a human colorectal carcinoma cell line and consequent enhancement of tumor antigen expression. Immunol Lett *36*: 179-185, 1993.

27 Dansky-Ullmann C, Salgaller M, Adams S *et al*: Synergistic effects of IL-6 and TNF-r on carcinoembryonic antigen (CEA) and HLA expression by human colorectal carcinoma cells: role for endogenous TFN-β. Cytokine *7*: 118-129, 1995.

28 Lahm H, Suardet L, Laurent PL *et al*: Growth regulation and co-stimulation of human colorectal cancer cell lines by insulin-like growth factor I, II and transforming growth factor α. Br J Cancer *65*: 341-346, 1992.

29 O'Brien S, Kantarjian H, Freireich E *et al*: CI-973, a new platinum derivative with potential anti-leukemic activity. Cancer Res *52*: 4130-4134, 1992.

30 Jeha S, Luo XN, Beran M *et al*: Antisense RNA inhibitor of phosphoprotein p18 expression abrogates the transformed phenotype of leukemic cell. Cnacer Res *56*: 1445-1450, 1996.
31 Cos S, Fernandez R, Guezmes A *et al*: Influence of melatonin on invasive and metastatic properties of MCF-7 human breast cancer cells. Cancer Res *58*: 4383-4390, 1998.
32 Brozek W, Bises G, Girsch T *et al*: Differentiation-dependent expression and mitogenic action of interleukin-6 in human colon carcinoma cells: relevance for tumor progression. Euro J Cancer *41*: 2347-2354, 2005.
33 Fukada T, Hibi M, Yamanaka Y *et al*: Two signals are necessary for cell proliferation induced by a cytokine gp 130: involvement of STAT 3 is antiapoptosis. Immunity *5*: 449-460, 1996.
34 Moran DM, Mayes N, Koniaris LG *et al*: Interleukin-6 inhibits cell proliferation in a rat model of hepatoccellular carcinoma. Liver International *25*: 445-457, 2005.
35 Spiotto MT and Chung TD: STAS 3 mediates IL-6 induced growth inhibition in the human prostate cancer cell line LN cap. Prostate *42*: 88-98, 2000.
36 Bellido T, O'Brien CA, Roberson PK and Manolagas SC: Transcriptional activation of the P21 (WAF 1, CIP 1, SPI 1) gene by interleukin-6 type cytokine. A prerequisite for their pro-differentiating and anti-apoptotic effects on human osteoblastic cells. J Biol Chem *273*: 21137-21144, 1998.
37 Shin SI, Freedman VH and Risser R: Tumorigenicity of virus-transformed cell in nude mice is correlated specifically with anchorage independent growth *in vitro*. Proct Natl Acad Sci USA *72*: 4435-4439, 1975.
38 Gui GP, Puddefoot JR, Vinson GP *et al*: Altered cell-matrix contact: a prerequisite for breast cancer metastasis? Br J Cancer *75*: 623-633, 1997.
39 Sommers CL, Byers SW, Thompson EW *et al*: Differentiation state and invasiveness of human breast cancer cell lines. Breast Cancer Res Treat *31*: 325-335, 1994.
40 Thompson EW, Torri J, Sabol M *et al*: Oncogene-induced basement membrane invasiveness in human mammary epithelial cells. Clin Exp Metastasis *12*: 181-194, 1994.
41 Chen Y, Pawlikowska L and Yao JS: Interleukin-6 involvement in brain anteriovenous malformation. Anu Neurol *59*: 72-80, 2006.
42 Yao JS, Zhai W, Young WL *et al*: Interleukin-6 triggers human cerebral endothelial cells proliferation and migration: the role for KDR and MMP-9. Biochem Biophys Res Commun *342*: 1396-1404, 2006.
43 Yang S, Hu S, Hsieh YC *et al*: Mechanism of IL-6-mediated cardiac dysfunction following trauma hemorrhage. J Mol Cell Cardiol *40*: 570-579, 2006.
44 Wung BS, Hsu MC, Wu CC and Hsieh CW: Resveratrol suppresse IL-6-induced ICAM-gene expression in endothelial cells: effects on the inhibition of STAT 3 phosphorylation. Life Science *78*: 389-397, 2005.
45 Wei LH, Kuo ML, Chen CA *et al*: Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis *via* a STAT 3 pathway. Oncogene *22*: 1517-1527, 2003.
46 Su JL, Lai KP, Chen CA *et al*: A novel peptide specifically binding to interleukin-6 receptor (gp 80) inhibits angiogenesis and tumor growth. Cancer Res *65*: 4827-4835, 2005.
47 Suzuki A, Hanada T, Mitsuyama K *et al*: CIS 3/ SOCS 3/ SSI 3 play a negative regulatory role in STAT 3 activation and intestinal inflammation. J Exp Med *193*: 471-481, 2001.
48 Yoshimura A, Mori H, Ohishi M *et al*: Negative regulation of cytokine signaling influences inflammation. Current Opin Immunol *15*: 704-708, 2003.
49 Sommer U, Schmid C, Sobota RM *et al*: Mechanisms of SOCS 3 phosphorylation upon interleukin-6 stimulation contribuations of Src-and receptor-tyrosine kinase. J Biol Chem *280*: 31478-31488, 2005.

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