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
volume 200 µl). After 4 days at 37°C and in a 5% CO2 atmosphere (except SW480), proliferation was assessed by 3-[4, 5-dimethylthiazol-2-yl]-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 5x104 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 (1x105 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% CO2. 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 compartment was then seeded with Matrigel-coated wells and incubated for 10 min, 30 min, or 60 min at 37°C in 5% CO2. The introduction of fibronectin into the lower compartment accelerated the movement of the cells from upper chamber through the collagen IV membrane separating the upper from the lower chambers. IL-6 (50 ng/ml but not other concentrations) significantly increased, SW-480 cells (5x104 cells per chamber) were added to the upper from the lower chambers. IL-6 (50 ng/ml but not other concentrations) significantly increased, SW-480 cells (5x104 cells per chamber) were added to the lower chamber. 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...
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 SW480. 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 SW480 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. LS1034 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 metastatic events.

**Table I. Summary of the changes of biological properties of SW480 after exogenous IL-6 was supplied.**

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

*when compared to control group, p<0.05.
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 \textit{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 \textit{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 \textit{et al.} reported that 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 expression.
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...
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
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.

Received September 1, 2006
Accepted October 5, 2006