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Differentiation-dependent expression and mitogenic action of interleukin-6 in human colon carcinoma cells: Relevance for tumour progression

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Abstract

Interleukin (IL)-6 mRNA expression in general is low in normal, adenomatous and cancerous human colon mucosa, except in rather undifferentiated lesions, in which IL-6 is overexpressed. Cytokeratin (CK) 8-positive carcinoma cells were identified by double immunostaining as almost exclusive source of IL-6. Likewise, in five (sub)clones of primary culture COGA-1 and COGA-13 human colon carcinoma cells, and in three established cell lines (Caco-2/AQ, Caco-2/15 and HT-29), efficient translation of IL-6 mRNA into protein was observed only in the least differentiated COGA-13 cells. Notably, IL-1β (5 ng/ml) enhanced IL-6 release in COGA-13 cultures by three orders of magnitude. Although all cell clones studied expressed the IL-6 receptor (IL-6R), rhIL-6 (1–100 ng/ml) had a significant effect on cellular proliferation only in highly differentiated Caco-2 cells. Our data imply that IL-6, when released from rather undifferentiated carcinoma cells, particularly in response to IL-1β, can advance tumour progression through paracrine growth stimulation of normal or highly differentiated colon tumour cells with intact STAT-3-mediated IL-6 signalling.

Keywords: Colon cancer; COGA cells; Caco-2 cells; Proliferation; Interleukin-1; Prostaglandins; IL-6 gene polymorphisms; IL-6 receptor-α; Gp130; STAT3

1. Introduction

Interleukin (IL)-6 is a pleiotropic immunoregulatory cytokine that plays an important role in tumour progression, metastasis and angiogenesis [1,2]. Although Shirota *et al.* [3] detected IL-6 immunoreactivity in tumour cells in the majority of human colon cancers and suggested that IL-6 could regulate neoplastic cell growth in an autocrine fashion, data on a possible relation between IL-6 production and tumour progression are conflicting. Komoda *et al.* [4] found an approximately eightfold increase of IL-6 protein in cancerous lesions when compared to normal colon mucosa, but failed to establish any correlation between IL-6 production and parameters of tumour progression. Kinoshita *et al.* [5] reported that in colon cancer patients, IL-6 serum concentrations correlated with tumour tissue concentrations and proliferative activity, but mean IL-6 levels were not significantly different in tumours of different stages. Also, Piancatelli *et al.* [6] detected IL-6 mRNA in cancerous tissue in 10 out of 12 cases of human colon cancer, but serum levels of IL-6 were independent of mRNA expression in tumour tissue and were not correlated with the histopathological and clinical parameters of the disease. These findings indicate that tumour cells

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in human colon cancers display a wide heterogeneity in their potential to express and produce IL-6.

This would also explain why several studies on IL-6 mRNA and protein levels in primary and established colon cancer cell lines yielded disparate results: Schneider *et al*. [7] noted that IL-6 is expressed in primary and metastatic colon cancer cell lines at the message though not at the protein level; Matsuo *et al*. [8] observed expression of IL-6 mRNA in only one out of nine established colorectal cancer cell lines; and in Caco-2 cell line no evidence was obtained for constitutive expression of IL-6 at the message or protein level [9,10]. However, IL-6 mRNA expression and translation into protein can be induced in Caco-2 cells by cytokines such as IL-1β, interferon-γ, and tumour necrosis factor-α [9–11].

Data on growth stimulatory effects of IL-6 on human colon carcinoma cells are also inconsistent. Schneider *et al*. [7] reported that IL-6 induced clonogenic growth of one human primary colon cancer cell line, whereas five others were totally unresponsive to the cytokine though they expressed both components of the membrane-bound IL-6 receptor (IL-6R): the IL-6-binding α-chain (IL-6Rα) and the signal transducing unit gp130 [1]. Similarly, studies by Yuan did not observe an effect of IL-6R-activated intracellular signalling on growth of human colon carcinoma cell lines RKO and 228 [12].

Since it is obvious that human colon tumour cells differ widely in their potential to produce IL-6 and to express the IL-6R complex, and consequently, to respond to an autocrine/paracrine growth regulatory action of IL-6, we wondered whether these differences could be attributed to changes in the degree of cellular differentiation during tumour progression. Therefore, we analysed mRNA expression of IL-6, IL-6Rα, and of gp130 in tissue specimens of normal human colon mucosa, adenomatous polyps and of colon cancers of different histological grades. We used immunocytochemistry to detect IL-6 protein in cancerous lesions. Furthermore, in a panel of eight colon carcinoma cell lines with each one showing a different degree of expression of the differentiation marker alkaline phosphatase, we studied basal and cytokine-stimulated IL-6 mRNA expression and protein release and determined the effect of the cytokine on cellular proliferation.

2. Materials and methods

2.1. Human colon tissue

Specimens of tumour tissue, of adjacent mucosa from the same patient, and of normal colon mucosa from diverticulitis patients after stoma re-operation were received from Hospital Rudolfstiftung, Vienna. Tumours were classified according to the TNM system of the AJCC/UICC [13]. For histopathological grading the following criteria were applied: G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; G4, undifferentiated.

2.2. Characterisation of human colon adenocarcinoma-derived cell clones

Primary colon adenocarcinoma cell clones designated COGA-1 and COGA-13, respectively, were isolated by Dr. Ernst Wagner and Alexandra Sinski at Boehringer Ingelheim Austria, Vienna, as described in detail elsewhere [14]. From the COGA-1 clone, which was originally derived from a Dukes' stage B, pT3, moderately differentiated (*i.e.*, G2) carcinoma, three sub-clones, designated COGA-1A, E and F, were established, which differed from each other in growth behaviour and differentiation, *i.e.* alkaline phosphatase activity (Table 1).

COGA-13 cells, which were derived from a stage pT2 carcinoma graded G3, exhibit only weak alkaline phosphatase activity (Table 1). In addition to the epithelial cell marker cytokeratin 8 (CK8), COGA-13 cells also express a high level of vimentin (not shown) indicating that they had undergone epithelial–mesenchymal transition.

Table 1
Proliferation and differentiation characteristics of human colon carcinoma cell clones

Clone	Doubling time (h)	Alkaline phosphatase (U/mg protein)	
	Log growth phase	Log growth phase	Confluence
Primary culture-derived cell clones			
COGA-1	42	4.1 ± 0.3	7.1 ± 0.6
COGA-1A	15	1.0 ± 0.1	2.9 ± 0.1
COGA-1E	42	2.8 ± 0.2	10.7 ± 0.6
COGA-1F	23	0.5 ± 0.1	4.8 ± 0.2
COGA-13	48	0.3 ± 0.1	0.7 ± 0.2
Cell line-derived clones			
Caco-2/AQ	24	20.3 ± 2.6	60.2 ± 9.1
Caco-2/15	36	25.0 ± 2.9	190.4 ± 21.8
HT-29	20	0.05 ± 0.02	0.09 ± 0.02

Two Caco-2 cell clones, which had been analysed previously for their proliferative potential and for their degree of differentiation [15] (see also Table 1), were used in the present study: clone Caco-2/15 was obtained from Dr. A. Quaroni, Cornell University, NY. From this cell line, we isolated the sub-clone Caco-2/AQ by dilution plating after 100 passages.

The human colon carcinoma cell line HT-29 was obtained from the ATCC repository. Proliferation and differentiation characteristics are given in Table 1.

2.3. Cell culture

Human colon cancer cells were routinely cultured in vented tissue culture flasks (Asahi Techno Glass Corporation, Iwaki Scitech division, Tokyo, Japan) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Culture medium was DMEM supplemented with 4.0 mM glutamine, 10% (v/v) fetal calf serum (FCS) (heat-inactivated at 56 °C for 30 min), 20 mM HEPES, 50 U/ml penicillin and 50 μg/ml streptomycin. Cultures were re-fed every 48 h and sub-cultured serially when approximately 80% confluent. Cells between passages 6 and 24 were cultured for indicated time periods in the absence or presence of one of the following treatments: 5 ng/ml IL-1β (ImmunoTools, Friesoythe, Germany); 10^{-6} M indomethacin, 10^{-7} M PGE_2 (both from Sigma); 0.01–100 ng/ml rhIL-6 (Strathmann Biotec AG, Hamburg, Germany); 10^{-6} M cucurbitacin I (Merck, Darmstadt, Germany); 1.0 μg/ml of a neutralising goat polyclonal anti-IL-6 antibody, or of a mouse monoclonal anti-IL-6Rα antibody (all from R&D Systems, Minneapolis, MN).

2.4. Evaluation of cellular growth and differentiation

Population doubling time during logarithmic growth was calculated for each cell clone from cell numbers determined in a Coulter Counter (model D, Coulter Electronics, Luton, UK). Degree of cellular differentiation and its changes related to transition in the post-confluent state were evaluated from activity of the marker enzyme, alkaline phosphatase, according to Stierum *et al.* [16]. The effect of rhIL-6 on cellular proliferation was determined by measurement of [^{3}H]thymidine incorporation into cellular DNA.

2.5. Determination of IL-6 protein

IL-6 concentrations in cell culture supernatants were determined by enzyme-linked immunosorbent assay (ELISA) (eBioscience, San Diego, CA) according to the protocol of the manufacturer.

2.6. Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted by using Trizol (GibcoBRL). 2 μg RNA was reverse transcribed with random hexamer primers using a cDNA synthesis kit (SuperScript™ II, Invitrogen). First-strand cDNA was amplified with primer pairs for IL-6, IL-6Rα, gp130, and for the epithelial cell marker CK8, respectively (MWG-Biotech AG, Ebersberg, Germany). To amplify a 349 base pair segment of IL-6 cDNA, the primer pairs used were 5′-TTC-AAT-GAG-GAG-ACT-TGC-CTG-3′ (sense) and 5′-ACA-ACA-ACA-ATC-TGA-GGT-GCC-3′ (antisense) [17]. The primer pairs used to amplify a 618 base pair segment of IL-6Rα cDNA were 5′-GTG-AGG-AAG-TTT-CAG-AAC-AGT-CCG-3′ (sense) and 5′-TGG-GAG-GCT-TGT-CGC-ATT-TG-3′ (antisense) [17]. The gp130 primer pairs were 5′-TAA-AGG-CAT-ACC-TTA-AAC-AAG-C-3′ (sense) and 5′-GTG-AAT-TCT-GGA-CCA-TCC-TTC-C-3′ (antisense) to amplify a 292 base pair segment [18]. Primer pairs for a 520 base pair segment of the CK8 gene were 5′-TGG-GCA-GCA-GCA-TTA-ACT-TTC-3′ (sense) and 5′-AGG-CGA-GAC-TCC-AGC-TCT-AC-3′ (antisense) [19]. Using the GeneAmp PCR System 9600 (Perkin–Elmer, Norwalk, CT), the thermocycling conditions began with 94 °C for 2 min followed by 34 cycles (IL-6, IL-6Rα, gp130) and 30 cycles (CK8), respectively, of: 94 °C 15 s, 62 °C 30 s, 72 °C 45 s, followed by 10 min at 72 °C. PCR products were separated and visualised on 2% agarose gels with ethidium bromide.

2.7. Immunohistochemistry

Immunohistochemical double staining for IL-6 and cytokeratin 8 (CK8) was performed on 5 μm cryosections fixed in 2% (w/v) paraformaldehyde. After washing, slides were incubated for 15 min with a 10% solution of FCS in PBS (v/v) with 0.05% Tween 20 (w/v) to block unspecific binding. IL-6 was detected with a goat polyclonal anti-human IL-6 antibody (R&D Systems, Minneapolis, MN) (20 μg/ml, 2 h incubation), followed by a FITC-labelled anti-goat antibody (Molecular Probes, Eugene, OR) (2 μg/ml, 1 h). CK8 was labelled with a mouse monoclonal anti-human CK8 antibody (Chemicon International, Temecula, CA) (5 μg/ml, 2 h). A Cy3-labelled anti-mouse antibody (Rockland, Gilbertsville, PA) (0.5 μg/ml, 1 h incubation) was used as secondary antibody. Sections were mounted under cover slips in Vectashield anti-fading medium (Vector Laboratories, Inc., Burlingame, CA) and examined under a Nikon Eclipse E400 fluorescence microscope.

2.8. Western blot analysis

STAT-3 protein was immunoblotted with a polyclonal rabbit anti-human STAT-3 antibody (Cell Signaling Technology, Inc., Beverly, MA). Secondary antibody was a horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibody (Amersham Biosciences, Buckinghamshire, UK), which was detected with the SuperSignal CL-HRP Substrate system (Pierce, Rockford, IL).

3. Results

3.1. Expression of IL-6, IL-6Rα and gp130 mRNA in normal and neoplastic human colon tissue

We used RT-PCR with CK8 as reference gene for semi-quantitative evaluation of IL-6, IL-6Rα and gp130 mRNA expression levels in epithelial cells in normal mucosa of non-cancer patients, in adenomas and in cancerous lesions of different grades and stages, as well as in the adjacent mucosa of the same cancer patients (Fig. 1). IL-6 mRNA expression was low in normal colon mucosa from non-cancer patients, but rose to four times as high levels in adenomatous polyps and in cancerous lesions graded G1–G2. A 20-fold higher than normal IL-6 gene activity was observed in undifferentiated G3-4 carcinomas. IL-6 message in the adjacent mucosa outside the tumour border on the average was elevated when compared to normal mucosa from non-cancer patients, but in any case was conspicuously lower than in the neighbouring cancerous lesion (Fig. 1(A)).

IL-6Rα and gp130 mRNA could be easily detected in normal colon mucosa cells (Fig. 1(B) and (C)). Cumulative data (Fig. 1(B) and (C)) showed no significant change in the expression of the two components of the IL-6 receptor complex on transition into the neoplastic state and during further progression through the adenoma/carcinoma sequence, except that IL-6Rα mRNA levels were significantly elevated in G3–G4 carcinomas (Fig. 1(B)).

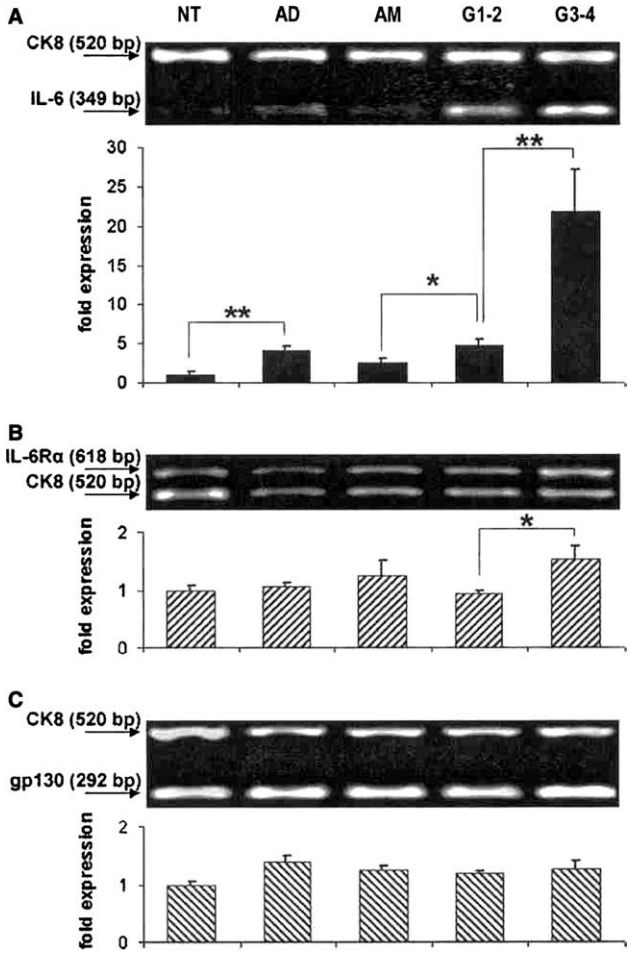

Fig. 1. mRNA expression of (A) interleukin (IL)-6, (B) IL-6 receptor (IL-6R)-α, and (C) gp130 in relation to epithelial cell marker cytokeratin 8 (CK8). NT, normal tissue; AD, adenomas; AM, adjacent mucosa; G1–G4, cancers of different grades. Representative reverse transcriptase polymerase chain reactions (RT-PCR) are shown above cumulative data in histograms (means ± SEM, $n = 4–8$; arbitrary densitometric units were normalised to CK8. Mean NT set to 1). Statistically significant differences: $^{*}P < 0.05$; $^{**}P < 0.01$ (Student's *t*-test).

3.2. IL-6 immunohistochemistry

Results from RT-PCR analysis of tumour tissue specimens of different grades had indicated that IL-6 gene activity is highest in rather advanced cancers (Fig. 1(A)). Accordingly, tissue specimens from low grade cancers, *i.e.*, G1–G2, stained only weakly, if at all, for IL-6, whereas clear evidence for the presence of IL-6 protein by immunofluorescence was obtained in rather undifferentiated cancers (Fig. 2). To get accurate information on the cellular localisation of IL-6, we used double immunostaining with fluorescent antibodies against IL-6 and the epithelial cell marker cytokeratin (CK) 8. Fig. 2 shows co-expression of IL-6 and CK8 in the tubular-villous structures of a G3 tumour. In addition, IL-6 specific immunostaining was also detected in some CK8-negative cells within the tumour stroma (Fig. 2(B)).

3.3. Expression of IL-6, IL-6Rα and gp130 mRNA in human colon carcinoma cell clones

The results of RT-PCR analysis of IL-6, IL-6Rα and gp130 mRNA expression in human colon carcinoma primary culture cell clones and in established cell lines are shown in Fig. 3. IL-6 expression levels were generally low in COGA-1 clones and in Caco-2 as well as HT-29 cell lines. In contrast, IL-6 message was abundant in COGA-13 cells. IL-6Rα and gp130 transcripts could be amplified from mRNA isolated from COGA-1 and COGA-13 clones as well as from Caco-2 and HT-29 cells.

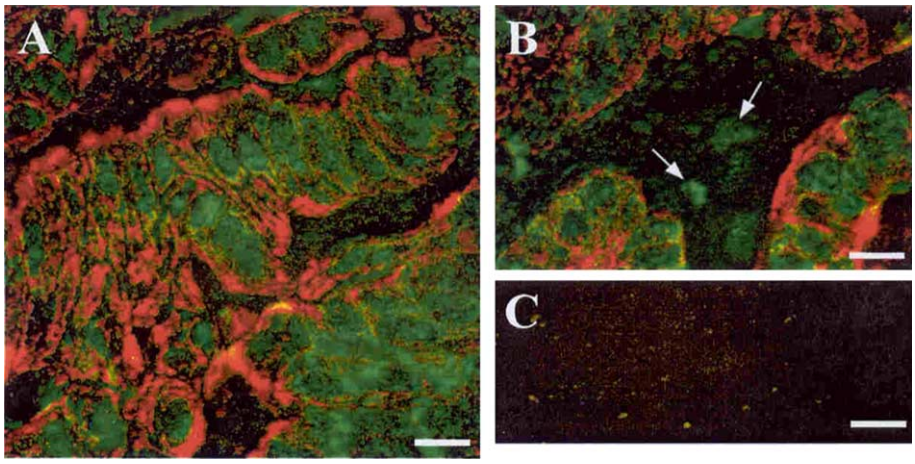

Fig. 2. Immunohistochemical double staining of cryosections of a G3 colon adenocarcinoma. (A) High-intensity immunostaining of IL-6 (green) in CK8-positive cells (red). (B) The presence of IL-6 in CK8-negative cells (arrows) within tumour stroma; (C) negative control. Scale bar = 15 μm.

3.4. IL-6 production by cultured human colon adenocarcinoma cell clones

Human colon adenocarcinoma primary cell clones and cell lines were cultured three days after confluence and the amount of IL-6 released into the culture medium within 24 h was measured by ELISA (Fig. 3). Without any addition to the culture medium, only the COGA-13 cells produced considerable amounts of IL-6. All other cell clones tested released, if at all, very little IL-6, so that cytokine concentrations in the medium at 24 h culture time were often relatively close to the detection limit of the assay (Fig. 3).

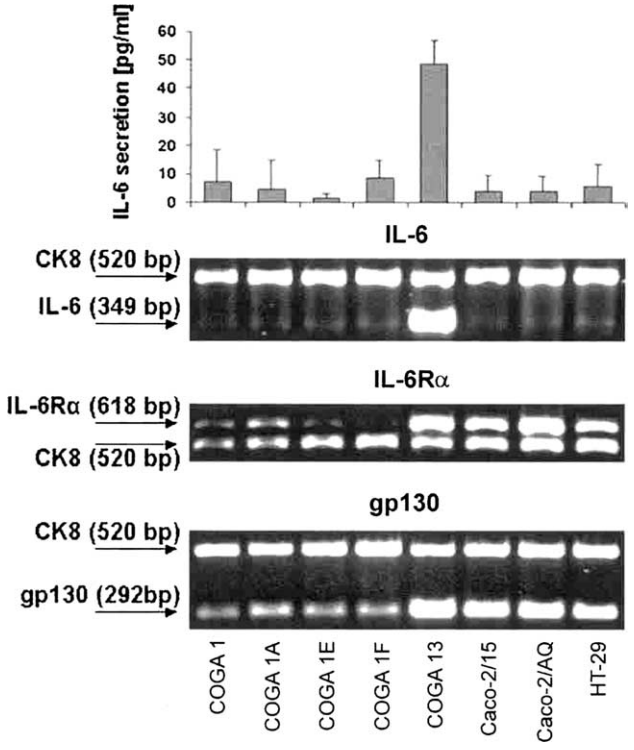

Fig. 3. Representative RT-PCR amplification of mRNA transcripts specific for IL-6, IL-6Rα and gp130 in human colon adenocarcinoma-derived cell clones. cDNA was diluted 1/10 before amplification of CK8, IL-6Rα and gp130 PCR products, RT-PCR for IL-6 was conducted without cDNA dilution. Histogram shows data from ELISA of basal IL-6 production during 24 h culture (means ± SD, $n = 5$).

PGE_2 and IL-1β, two potent stimulators of *IL-6*, are either produced by colon carcinoma cells [20] or, respectively, released from tumour-infiltrating leukocytes during a host-immune response. In the next series of experiments, we therefore tested the effect of these two agents on *IL-6* expression and protein synthesis in Caco-2/AQ cells as example for highly differentiated colon carcinoma cells, and in COGA-13 cells, which exhibited a rather low degree of differentiation (see Table 1). Results are shown in Fig. 4. Constitutive IL-6 mRNA expression was below detection in Caco-2/AQ cells in this experiment, but high in COGA-13 cells in relation to the epithelial cell marker CK8. Accordingly, an approximately tenfold difference between Caco-2/AQ and COGA-13 cells was observed in respect to their basal IL-6 production rate (see also Fig. 3). Blocking of endogenous PG synthesis by 10^{-6} M indomethacin did not affect IL-6 mRNA expression in both cell clones, a result which was also reflected at the protein level (Fig. 4). Addition of 10^{-7} M PGE_2 had no effect on *IL-6* in Caco-2/AQ cells, but raised expression and production of the cytokine in COGA-13 cells (by 75%). IL-1β (5 ng/ml) up-regulated IL-6 mRNA in both cell clones and this brought about an increase in IL-6 protein release, which was threefold in Caco-2/AQ cells, but, most notably, up to 1000-fold in COGA-13 cells. These effects of IL-1β were fully preserved in the presence of 10^{-6} M indomethacin (Fig. 4).

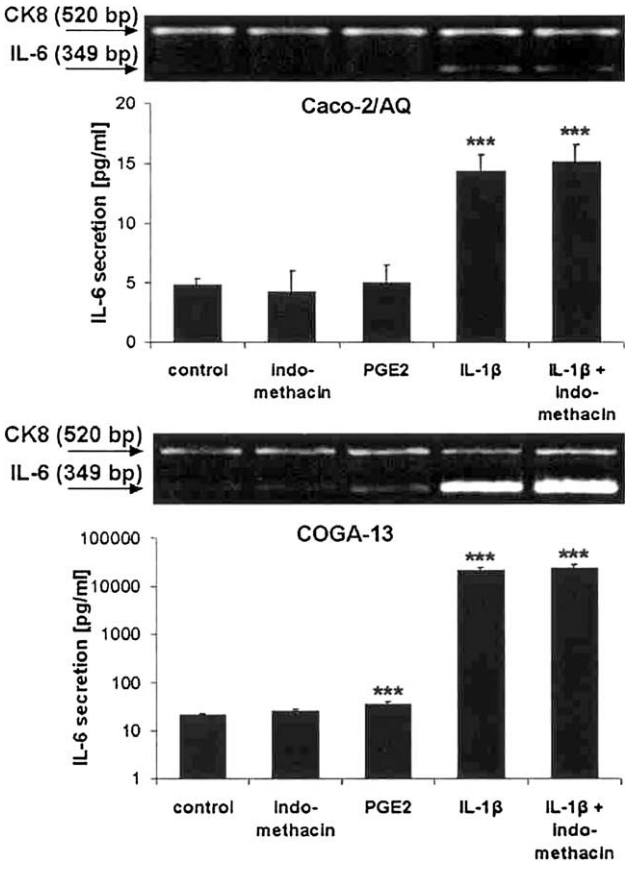

Fig. 4. Effect of prostaglandin (PG) E_2 (10^{-7} M) and IL-1β (5 ng/ml) on mRNA (inserts; incubation 4 h) and protein expression (histograms; incubation 24 h) of IL-6 in Caco-2/AQ and COGA-13 cells. Data are shown as means ± SD, $n = 4$. Statistically significant difference from controls: $^{***}P < 0.001$ (Student's *t*-test).

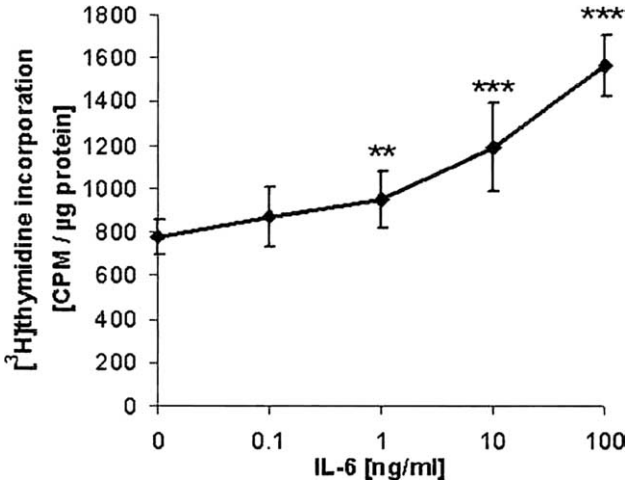

Fig. 5. Concentration-dependence of the proliferative effect of rhIL-6 on confluent Caco-2/AQ cells. Statistically significant difference from controls: $^{**}P < 0.01$; $^{***}P < 0.001$ (Student's *t*-test).

3.5. Effect of IL-6 on proliferation of human colon carcinoma cell clones

Since all cell clones of the present study exhibited expression of the IL-6 receptor complex (see Fig. 3), we evaluated a possible effect of IL-6 on their cellular proliferation. IL-6 was added to colon carcinoma cell cultures in the 0.01–100 ng/ml concentration range at two different time points, *i.e.*, 3 days after seeding, when cells were subconfluent and still in the logarithmic growth phase, and 3 days after cells had become confluent and had thus entered the resting phase. In subconfluent cultures, IL-6 had no effect on proliferation as assayed by [^{3}H]thymidine labelling of cellular DNA in any of the cell lines (not shown). After confluence, IL-6 stimulated cell division of Caco-2/AQ in a concentration-dependent manner between 1 and 100 ng/ml (Fig. 5). The proliferative effect of IL-6 was completely abolished, however, when signalling was blocked by inhibition of STAT-3α phosphorylation with 10^{-6} M cucurbitacin I (not shown). In Caco-2/15 cells, the cytokine stimulated proliferation by approximately 50% ($P < 0.001$) though only at 100 ng/ml. None of the other confluent cell clones responded to IL-6 with a significant change in their growth rate (data not shown).

To test the possibility that COGA-13 cells were insensitive to exogenous IL-6 because the IL-6R was occupied by IL-6 released into the culture medium, we measured [^{3}H]thymidine incorporation in COGA-13 cells that had been cultured for 72 h in the presence of a neutralising IL-6 antibody, or, respectively, of a receptor-blocking IL-6Rα antibody. Though both antibodies were added at an excess concentration of 1.0 µg/ml, no effect on growth of COGA-13 cells was observed (data not shown).

3.6. Human colon carcinoma cell clones express STAT-3

We performed Western blot analysis of STAT-3 protein in selected human colon carcinoma cells to test the possibility that their differential response to rhIL-6 was due to cell clone-specific differences in STAT-3 expression. Fig. 6 shows that in Caco-2/AQ cells, as expected, but also in IL-6-resistant COGA-1A and COGA-13 cells the functional STAT-3α isoform was highly abundant in

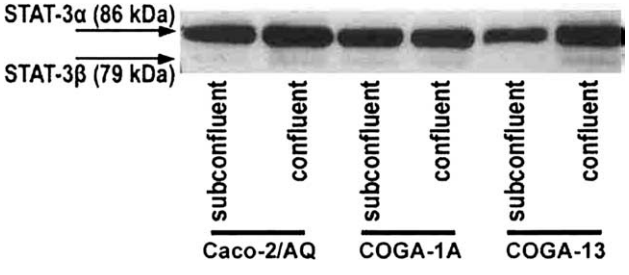

Fig. 6. Immunoblot of STAT-3α and STAT-3-β isoforms in human colon carcinoma cells. Samples were separated by 12% SDS–polyacrylamide gel electrophoresis with 15 µg total protein per lane.

both the subconfluent and confluent state. Only low amounts of the STAT-3β splice variant were detected.

4. Discussion

Combined results from the present study provide evidence that *IL-6* gene activity is low in normal human mucosa and rises only moderately during progression from adenomatous to low grade, *i.e.*, G1 to G2, cancerous lesions (Fig. 1). Accordingly, we could not detect any significant immunohistochemical staining for IL-6 protein in well to moderately differentiated carcinomas. In contrast, high levels of IL-6 were observed in rather undifferentiated lesions (G3–G4) (Fig. 1), and were effectively translated into IL-6 protein so that tumour cells stained uniformly positive with high signal intensity with an IL-6-specific fluorescent antibody (Fig. 2).

Our investigation on IL-6 expression by primary culture cell clones and established carcinoma cell lines (Fig. 3) brought further support for the notion that IL-6 is produced to a considerable extent only by carcinoma cells exhibiting a low degree of differentiation. Although each of the cell clones studied expressed *IL-6*, considerable amounts of IL-6 protein were released only into cultures of the COGA-13 clone, which was derived from a lesion graded G3, and accordingly, exhibited the lowest level of alkaline phosphatase activity of all primary culture clones studied (Table 1). Moreover, COGA-13 cells express high levels of vimentin (data not shown), an indication of transition from epithelial to the mesenchymal state.

A similar pattern of differentiation-dependent IL-6 production apparently exists also in human ovarian cancer: Hefler *et al.* [21] analysed the frequency of a common G/C polymorphism at position −174 of the IL-6 gene and found that at an early tumour stage ovarian cancer cells expressed predominately –174C alleles, whereas at later stages the −174G polymorphism prevailed. Interestingly, −174C *IL-6* is less efficiently translated into protein than its −174G counterpart [22]. This may be the reason why Belluco *et al.* [23] found that colon cancer patients carrying the −174G polymorphic *IL-6* gene had significantly higher IL-6 serum levels than patients with the −174C genotype.

It is conceivable that also the striking difference in the regulation of transcriptional activity of *IL-6* in the Caco-2/AQ and COGA-13 cell clone is due to expression of two different *IL-6* genotypes. As shown in Fig. 4, in Caco-2/AQ cells, *IL-6* gene activity is rather insensitive to regulation by PGE_2 and by IL-1β. In contrast, COGA-13 cells harbour an *IL-6* gene variant, which is moderately up-regulated by PGE_2 but is highly responsive to IL-1β (Fig. 4). It remains to be seen whether this pattern of transcriptional regulation can be attributed also to the −174G polymorphism, or whether the extreme sensitivity to IL-1β is the result of positive co-operativity with other promoter variants as suggested by Terry *et al.* [24].

We had assumed that in case of overexpression of *IL-6* (Figs. 1–3), particularly when associated with high sensitivity to IL-1β, as observed in COGA-13 cells (Fig. 4), enough IL-6 can be produced by tumour cells for an efficient autocrine/paracrine stimulatory action on cell growth. This seemed to be even more likely because expression of IL-6Rα and of gp130 was retained at a normal level during tumour progression (Fig. 1). However, we observed a rather selective responsiveness of colon cancer cells to the growth modulating action of IL-6. Although all cell clones investigated expressed IL-6Rα and gp130 mRNA (Fig. 3), only in differentiated Caco-2 cell cultures, rhIL-6 stimulated proliferation (Fig. 5), whereas rather undifferentiated HT-29 cells and particularly all primary culture cell clones were completely resistant to the proliferative action of the cytokine (data not shown). There are several ways to explain this phenomenon: first, although we observed that average expression levels of IL-6Rα are elevated in late stage cancers (Fig. 1), we cannot exclude that, as suggested by Schneider and colleagues [7], in some of the tumour cell clones investigated IL-6Rα mRNA is not translated into protein; and second, one has to bear in mind that control of intestinal cell proliferation by IL-6 not only requires the expression of a functional IL-6R but also its coupling to the JAK/STAT-1/3 proliferative intracellular pathway [12,25–28] and phosphorylation of STAT-3α. Intracellular signalling is apparently blocked in COGA-1A and COGA-13 cells, which though exhibiting constitutive expression of STAT-3α protein (Fig. 6) nevertheless do not respond to IL-6 with an increase in growth rate. In contrast, IL-6 proliferative signalling is apparently intact in Caco-2 cells, as they express STAT-3 (Fig. 6) and respond to IL-6 like normal colonocytes by an increase in the rate of cell division (Fig. 5), which notably is abolished when STAT-3 activation is blocked by cucurbitacin I (not shown).

We conclude from our data that there exist two clinically relevant situations in which IL-6 could play a role in colon tumour development and progression: first, since signalling from IL-6R is transduced into STAT-3 activation in normal and highly differentiated neoplastic cells, a paracrine mitogenic action of IL-6 is responsible not only for epithelial cell hyperproliferation in ulcerative colitis [29] but also for subsequent progression into colorectal cancer; and second, IL-6 may be particularly effective in advancing the adenoma/carcinoma sequence in individuals carrying an IL-6 gene variant with high sensitivity to IL-1β. These high-risk patients are conceivably the prime candidates for a future adjuvant immune therapy with monoclonal anti-IL-6 or anti IL-6R antibodies.

Conflict of interest statement

None declared.

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