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Downregulation of wild-type β-catenin expression by interleukin 6 in human hepatocarcinoma HepG2 cells: a possible role in the growth-regulatory effects of the cytokine?

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Abstract

We investigated the antitumour effects of interleukin 6 (IL-6) on hepatocarcinoma HepG2 cells, endowed with high levels of a mutated, non-degradable, β-catenin. IL-6 produced minimal growth-inhibitory effects and no apoptosis or gross changes in cell adhesion. Interestingly, however, it caused a consistent decrease in the cytoplasmic levels of wild-type, but not of mutated, β-catenin protein. There was no effect on E-cadherin or γ-catenin and a reduction in α-catenin occurred only at high concentrations. IL-4, a non-related cytokine, did not modify the content of β-catenin. IL-6 did not influence β-catenin mRNA levels. LiCl, a potent inhibitor of Glycogen Synthase Kinase 3β (GSK3β) activity, abrogated the IL-6-induced inhibition of wild-type β-catenin. This indicates that IL-6 can affect wild-type β-catenin through a post-trascriptional mechanism, probably involving degradation of the protein. This effect might be related to the growth-regulatory activities of IL-6 in other situations, but can not counteract the oncogenic expression of mutated β-catenin in HepG2 cells or possibly in other tumour cells with similar gene mutations.

Keywords: Interleukin 6 (IL-6); Hepatic carcinoma; Cell growth; β-Catenin; Lithium chloride; Glycogen Synthase Kinase 3 β (GSK3β)

1. Introduction

Interleukin 6 (IL-6) is an inflammatory cytokine that induces a variety of biological responses and influences the growth of several target cells. It may be an exogenous or autocrine growth factor for various tumours, including multiple myeloma and prostate cancer [1–4], but may also exert inhibitory effects on other tumour types [5–7]. In addition, in melanomas, IL-6 seems to undergo a transition from a paracrine growth inhibitor to an autocrine stimulator during malignant progression [8].

Hepatic carcinoma is a relatively common tumour which results in a high mortality and often a poor response to present drug therapies [9]. Clearly, a further understanding of the biological basis of this tumour might suggest new strategies for its treatment. Interestingly, while earlier reports suggested that IL-6 inhibits the growth of both normal and malignant hepatocytes, more recent data have indicated that the cytokine may support hepatocyte proliferation and have a major role in liver regeneration after partial hepatectomy or toxic insults [10-12]. It may also favour the development of liver adenomas [12]. Nevertheless, it is not clear whether IL-6 exerts any direct influence on the growth of established hepatic tumours. To study this aspect, we have focused on the human hepatic carcinoma HepG2 cell line [13]. This cell line has been frequently used as a model for the study of IL-6-induced acute phase protein expression by liver cells and to dissect IL-6 signalling [14]. HepG2 cells have an aberrant expression of β-catenin [15,16]. β-Catenin links E-cadherin to the actin cytoskeleton via α-catenin and is essential for cadherin-mediated cell adhesion. However, there is emerging evidence that this protein may play an important role also in the processes of cell growth and survival by acting as a signal transducer of the Wnt/Wingless

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signalling pathway. Free β-catenin accumulates in response to Wnt signalling and binds to leukaemia enhancing factor (LEF)/T cell factor (TCF) transcription factors to modulate the activity of target genes involved in the control of cell proliferation and death [17,18]. When this signalling pathway is activated inappropriately it can lead to malignant transformation [19]. Oncogenic mutations of β-catenin or of its regulatory factors adenomatous polyposis coli (APC) or axin have also been found in other human liver cancer cell lines, clinical samples of hepatic carcinomas and many other tumour types [15,20–23].

In HepG2 cells, there is a large deletion (amino acids 25–140) of β-catenin that impedes the Glycogen Synthase Kinase 3β (GSK3β)-mediated phosphorylation of the protein at serine and threonine residues in the N-terminus region. The, otherwise short-lived, protein is thus prevented from being degraded through the ubiquitin–proteasome pathway [15,24–26] and accumulates in the nuclei thereby increasing specific LEF/TCF-mediated transcription [15,23]. In addition, the mutated β-catenin of HepG2 cells lacks the binding site of α-catenin, thus probably affecting E-cadherin-mediated cell adhesion [15].

In this report, we investigated the influences of IL-6 on the proliferation, survival and adhesion of HepG2 cells, as well as its effects on β-catenin. The effects of IL-6 were compared with those of interleukin 4 (IL-4), a non-related cytokine which is also able to interact with HepG2 cells [27].

2. Materials and methods

2.1. Tumour cells and treatments

HepG2 cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 (HyClone Europe Ltd, Cramlington, UK) supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin and 100 μg/ml streptomycin (all reagents were from HyClone Europe). Cells were grown as adherent cells in a humidified atmosphere at 37°C in 5% CO_2. They were subcultured after trypsinisation (with 0.05% (w/v) trypsin/0.02% (w/v) ethylenediamine tetraacetic acid (EDTA) in phosphate-buffered solution (PBS), from HyClone Europe) for 4 min at room temperature, washing and re-suspension in complete medium.

Recombinant human IL-6 and IL-4 were obtained from Insight Biotechnology LTD, Wembley, UK. Lithium chloride was from Sigma-Aldrich Srl, Milan, Italy. To test the effects of the agents, the cells were plated as described below and then incubated overnight. At time 0, the agents were added and the application of IL-4 and IL-6 was repeated after 48 h.

2.2. Assay of cell surface IL-6-receptor α (IL-6Rα) and glycoprotein (gp) 130 by flow cytometry

Briefly, cell suspensions were washed with PBS containing 1% (w/v) bovine serum albumin (BSA) and incubated on ice for 40 min with 1 μg of anti-human IL-6Rα (B-N12, IgG_1k, Biosource, Camarillo, CA, USA) or anti-human gp130 (AM64, IgG_1k, phycoerythrin (PE)-conjugated, Pharmingen, San Diego, CA, USA) monoclonal antibodies (MAbs) diluted in 100 μl of PBS/BSA. The reactivity of the anti-IL-6Rα MAb was detected with fluorescein isothiocyanate (FITC)-conjugated goat polyclonal antibodies to mouse immunoglobulin (Pharmingen; 1.2 μg in 100 μl of PBS/BSA). Washes with PBS/BSA were performed between each incubation. Fluorescence was analysed by flow cytometry using a fluorescence activated cell sorter (FACSort) instrument (Becton Dickinson, Montain View, CA, USA). The results were analysed using CellQuest™ software (Becton Dickinson) by subtracting the cells stained with the FITC-conjugated goat polyclonal antibodies to mouse immunoglobulin alone or negative isotype-matched PE-conjugated immunoglobulins (Pharmingen; 1 μg in 100 μl of PBS/BSA) from the cell population stained with the antibodies recognising IL-6Rα or gp130, respectively.

2.3. Soluble IL-6Rα or IL-6 enzyme-linked immunosorbent assay (ELISA)

Soluble IL-6Rα released by cells was measured by the Quantikine human IL-6 sR (soluble IL-6Rα) immunoassay (R&D Systems, Minneapolis, MN, USA). The presence of intracellular and extracellular human IL-6 was determined using a high-sensitivity ELISA kit (Amersham, Little Chalfont, UK). With this assay, the minimum detectable concentration of human IL-6 is 0.1 pg/ml.

Briefly, 5×10^5 HepG2 cells were incubated in complete medium. After 48 h of incubation, the culture medium of the cells was collected to measure the extracellular content of soluble IL-6Rα or of IL-6. The cells were also harvested by treatment with trypsin-EDTA and pelleted. The pelleted cells were resuspended and sonicated. After centrifugation, the medium was collected to determine the intracellular IL-6 concentration. Neither the soluble IL-6Rα nor the IL-6 immunoassay cross-reacted with the components of the complete medium used for the cultures.

2.4. Cytotoxicity and cell adhesion assays

Cytotoxicity was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye reduction assay. HepG2 cells were resuspended at 5×10^4 cells/ml in complete medium and 200 μl of cell suspension were distributed into each well of 96-well

microtitre plates (Techno Plastic-Products, Trasadingen, Switzerland). After different periods of treatment, 20 μl of MTT (Sigma-Aldrich Srl) working solution (5 mg/ml) were added to each culture well and the plates were incubated for 4 h at 37°C in a humidified 5% CO_2 atmosphere. The culture medium was removed from the wells and replaced with 100 μl of isopropyl alcohol supplemented with 0.05 N HCl. The absorbance of each well was measured with a microculture plate reader model 550 (Bio-Rad, Milan, Italy) at 540 nm. Cell survival was expressed as a percentage of the absorbance measured in the untreated cells.

To assay cell adhesion, HepG2 cells were suspended in complete medium at 5×10^4 cells/ml. One or 4 ml of the cell suspension were distributed into the wells of 24-well polypropylene plates (Techno Plastic-Products AG) or in 47 mm Petri polystyrene dishes (Sigma-Aldrich), respectively. This was done to provide the cells with different attachment substrates. The cells were incubated with various concentrations of IL-6 for up to 6 days. At various time intervals, the free floating cells and the adherent cells removed by trypsinisation were counted separately by the trypan blue dye exclusion test.

2.5. Evaluation of apoptosis by flow cytometry

Attached cells were treated with trypsin-EDTA and pelleted together with floating cells. Cells were washed twice with ice-cold PBS and then resuspended at 1×10^6/ml in a hypotonic fluorochrome solution containing propidium iodide 50 μg/ml in 0.1% (w/v) sodium citrate plus 0.03% (v/v) Nonidet P-40. After 1 h of incubation in this solution, the samples were filtered through nylon cloth, 40 μm mesh, and their fluorescence was analysed as single-parameter frequency histograms. The data were analysed with CellQuest™ software. Apoptosis was determined by evaluating the percentage of events accumulated in the $preG_o$–G_1 position, according to Ref. [28]. In some experiments, apoptosis was detected also by evaluating phosphatidylserine exposure on the surface of the cells after labelling with FITC-conjugated annexin V (Pharmingen) [29].

2.6. Western blot analysis

Mouse mAb (HECD-1) against human E-cadherin was obtained from Takara Shuzo Co., Shiga, Japan. Mouse MAbs against human α-, β- and γ-catenin were purchased from Transduction Laboratories, Lexington, KY, USA. Mouse MAb (AC-15) against β-actin was obtained from Sigma-Aldrich Srl. Anti-β-actin antibodies have been extensively used in Western blot analyses as epithelial loading controls and in our case this control was suitable since the β-actin molecule, which has a molecular mass of 42 kDa, migrates far from all the molecules we analysed.

Cells were washed three times with PBS and then lysed for 20 min on ice in hypotonic buffer containing 10 mM tris(hydroxymethyl)aminomethane TRIS, pH 7.4, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulphonyl fluoride (all from Sigma-Aldrich Srl). The cells were then mechanically disrupted by 30 strokes in a Dounce homogeniser. Cell lysates were centrifuged at 500*g* for 10 min at 4°C. The resulting supernatants were centrifuged at 100 000*g* for 1 h at 4°C. Aliquots of the whole cell lysates, pellets (membranes) or supernatants (cytoplasmic fraction) of the 100 000*g* centrifugation were subjected to sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS-PAGE), as previously described [16]. Proteins were electrophoresed onto Hybond-P membrane (Amersham) using a semi-dry fast blot apparatus (Bio-Rad). Membranes were blocked with 3% BSA in PBS–0.1% (v/v) Tween 20 (PBST) for 1 h and then probed with the MAbs for 1 h at 37°C. Anti-α-, β-, γ-catenin and -β-actin MAbs were diluted 1:100, 1:250, 1:1500 and 1:5000, respectively, in PBST containing 1% BSA. The concentration of the anti-E-cadherin MAb was of 10 μg/ml in the same medium. Blots were rinsed with PBST and incubated for 1 h at room temperature with peri-oxidase-conjugated secondary antibodies (Amersham) diluted 1:1000 in PBST containing 1% BSA. After rinsing with PBST, hybridisation was visualised using an enhanced chemiluminescence detection kit (Amersham). Different exposure times were used to optimise the signal for each type of hybridised bands. Relative estimates of bands were carried out by means of a GS-670 Bio-Rad Imaging Densitometer using the Molecular Analyst™/PC image analysis software (version 1.1). The signals obtained from the each experimental point were normalised to the signal obtained from β-actin.

2.7. Isolation of mRNA and reverse transcription-polymerase chain reaction (RT-PCR)

Cells were collected and centrifuged. $PolyA^+$-mRNA was extracted from frozen cells using a QuickPrep mRNA purification kit (Amersham). RT-PCR were then performed using the one-step protocol of the Ready-to-go RT-PCR beads kit (Amersham). Quantification and equalisation of the amount of cDNA was achieved using primers to amplifying glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. Briefly, equal volumes of mRNA preparation from IL-6-treated and untreated cells, the first-strand oligo(dT) primer and the appropriate set of oligonucleotide primers for β-catenin or GADPH were added individually to each dissolved bead in a total volume of 50 μl. First strand cDNAs were obtained after 30 min at 42°C. Following inactivation at 95°C for 5 min, PCR amplification was performed under the following reaction conditions: 95°C for 1 min, 58°C for 1 min, 72°C

for 1 min and a final extension at 72°C for 8 min. To amplify β-catenin and GAPDH fragments 20, 25 and 30 cycles were used to determine whether the DNA amplification was linear. All PCR products (10 μl) were analysed by electrophoresis on a 1.5% (w/v) agarose gel and photographed. Densitometric analysis of the bands was carried out as described in the previous section. The sequences of primers used in the RT-PCR were as follows: β-catenin: 5′-GTTTTGAAAATCCAGCGTGG-3′ (sense) and 5′-TTGAAGGCAGTCTGTCGTAA-3′ (antisense); GAPDH: 5′-TGACATCAAGAAGGTGGTGA-3′ (sense) and 5′-TCCACCACCCTGTTGCTGTA-3′ (antisense).

3. Results

3.1. Expression of IL-6Rα, gp130 and IL-6

Two transmembrane glycoproteins that form the functional IL-6 receptor have been identified and cloned: the ligand-binding receptor IL-6Rα and the signal transducer gp130. IL-6 promotes the sequential assembling of a multisubunit complex by binding first to IL-6Rα with a relatively low affinity. The high-affinity binding of the IL-6/IL-6Rα complex to gp130 is crucial for signal transduction, which proceeds by activation of tyrosine kinases of the Janus kinase (JAK) family [30].

It has been reported that in HepG2 cells high-affinity sites are present which bind IL-6 with an apparent K_D of 21 pmol/l [31]. Our flow cytometry immunofluorescence analysis specified that on the surface of the cells there is a more limited expression of IL-6Rα than of gp130 (Fig. 1). It is also known that IL-6Rα can bind IL-6 and mediate IL-6 function also in its soluble form [30]. ELISA assays showed that HepG2 cells release low, but measurable (60.1±0.4 pg/ml/48 h/5×10^5 cells), amounts of soluble IL-6Rα. We then examined whether HepG2 cells produce and secrete IL-6, but a sensitive ELISA assay did not detect the cytokine in the lysates or in the supernatants of the cultured cells (data not shown).

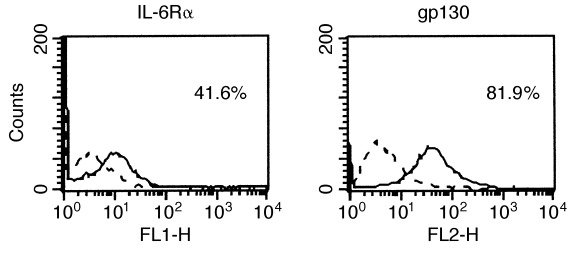

Fig. 1. Representative example of flow cytometry analysis of the expression of IL-6Rα or gp130 on the surface of HepG2 cells. The percentages of positive cells are indicated in each panel. Two repeat experiments gave very similar results.

Table 1
Effect of IL-4 or IL-6 on the growth of HepG2 cells

	72 h	144 h
IL-4 0.01 ng/ml	99.7±2.6	99.4±3.3
IL-4 0.5 ng/ml	88.1±4.2	97.6±2.5
IL-4 1 ng/ml	87.8±3.7	96.1±3.9
IL-4 3 ng/ml	87.7±3.4	95.1±4.1
IL-4 10 ng/ml	87.8±3.3	96.4±3.8
IL-4 30 ng/ml	84.7±2.7	96.9±2.2
IL-4 50 ng/ml	81.0±3.1	98.9±2.9
IL-6 0.01 ng/ml	101.3±2.4	99.3±1.6
IL-6 0.5 ng/ml	97.4±2.2	98.9±2.3
IL-6 1 ng/ml	98.9±3.6	96.2±2.6
IL-6 3 ng/ml	90.3±2.3	93.0±0.7
IL-6 10 ng/ml	86.7±1.9	90.8±2.1
IL-6 30 ng/ml	87.2±2.3	87.7±2.8
IL-6 50 ng/ml	91.0±3.5	93.8±3.7

The cells were incubated with IL-4 or IL-6 for the indicated times and then cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Data are expressed as per cent of untreated cells and are the means±standard deviation (S.D.) of three separate experiments, each of which was performed in quadruplicate.

3.2. Cytotoxicity, apoptosis and cell adhesion

HepG2 cells in exponential growth have a doubling time of 27.1 h. Treatment with IL-6 for 72 or 144 h induced a marginal (~10%) inhibition of the growth of HepG2 cells (Table 1). It did not significantly change their rate of apoptosis, which was detected either by staining the DNA with propidium iodide (Fig. 2) or by assessing the phosphatidylserine exposure on the surface of the cells (data not shown). At the same time points, IL-6 at concentrations of up to 50 ng/ml did not appear to grossly modify the adhesive properties of HepG2 cells, since it did not induce any change in the percentage of, either alive or dead, floating cells with respect to

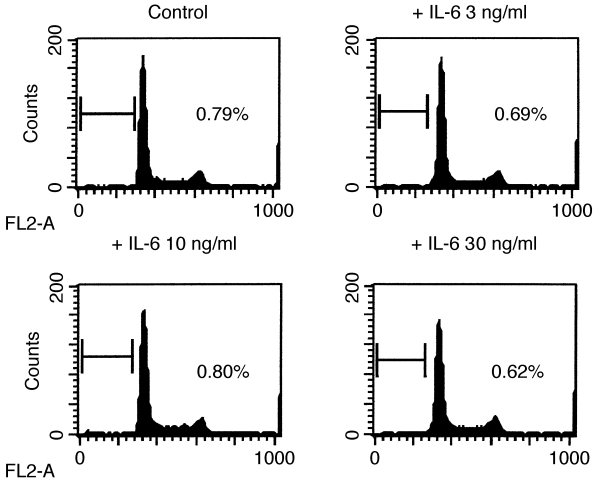

Fig. 2. Representative example of flow cytometry analysis of apoptosis in HepG2 cells treated for 144 h with IL-6. The percentages of events accumulated in the preG$_o$–G$_1$ position are indicated in each panel. Two repeat experiments gave very similar results.

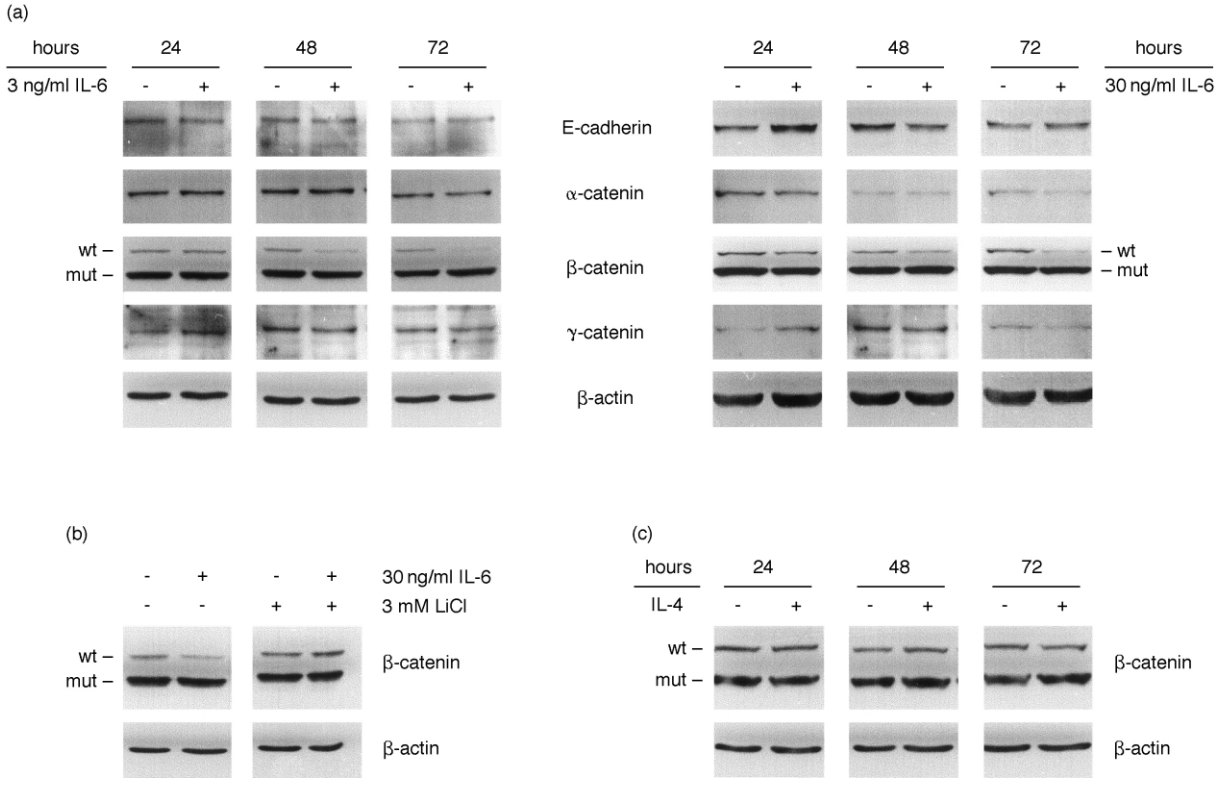

Fig. 3. Analysis of protein expression of E-cadherin and catenins in whole cell lysates of HepG2 cells: (a) left side: cells incubated for 24, 48 or 72 h without (−) or with (+) 3 ng/ml IL-6; right side: cells incubated for 24, 48 or 72 h without (−) or with (+) 30 ng/ml IL-6; (b) cells incubated for 72 h without (−) or with (+) 30 ng/ml IL-6 and without (−) or with (+) 3 mM lithium chloride; (c) cells incubated for 24, 48 or 72 h without (−) or with (+) 30 ng/ml IL-4. Wt and mut indicate, the wild-type and mutant form of β-catenin, respectively present in the HepG2 cells. The photographs shown are representative of the different exposure times used to visualise the bands and of three independent experiments. For further details, see Materials and Methods.

cells adherent to different, i.e. polypropylene or polystyrene, culture layers (data not shown).

Treatment with IL-4 caused a slight inhibition (~15%) of the growth of HepG2 cells after 72 h, but not 144 h of treatment (Table 1).

3.3. Effect of IL-6 on β-catenin protein and mRNA cell levels

HepG2 cells express high levels of a mutated 73 kDa β-catenin [15,16]. In comparison with the normal hepatocyte Chang liver cell line, they show also decreased levels of wild-type β-catenin (92 kDa), α- and γ-catenin [16]. Western blotting analyses on whole cell lysates showed that at 48 or 72 h after treatment with IL-6 (3 or 30 ng/ml) HepG2 cells undergo evident decreases (to ~40% of the control) in the levels of wild-type β-catenin, with no or minimal changes in those of mutated β-catenin, E-cadherin and γ-catenin (Fig. 3a). α-Catenin was decreased (to 40% of the control) after treatment for 72 h with the higher concentration of IL-6 tested (Fig. 3a). IL-4 did not exert any influence on the expression of either wild-type or mutated β-catenin in the HepG2 cells (Fig. 3c).

Further determinations on cell fractions demonstrated that wild-type β-catenin is decreased by IL-6 at the cytoplasmic level (Fig. 4), with no change in the cell membrane fraction (data not shown). Cotreatment with lithium chloride, a potent inhibitor of GSK3β activity [32], abolished the IL-6-induced decrease of wild-type β-catenin (Fig. 3b). However, it was not possible to test the effect with respect to other proteasome inhibitors

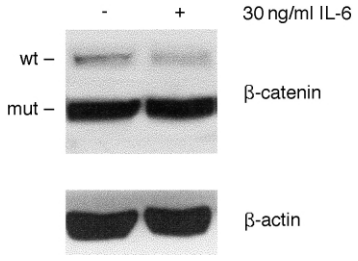

Fig. 4. Analysis of protein expression of β-catenin in the cytoplasmic fraction of HepG2 cells incubated for 72 h without (−) or with (+) 30 ng/ml IL-6. Wt and mut indicate, the wild-type and mutant form of β-catenin, respectively, present in the HepG2 cells. The photographs shown are representative of the different exposure times used to visualise the bands and of three independent experiments. For further details, see Materials and Methods..

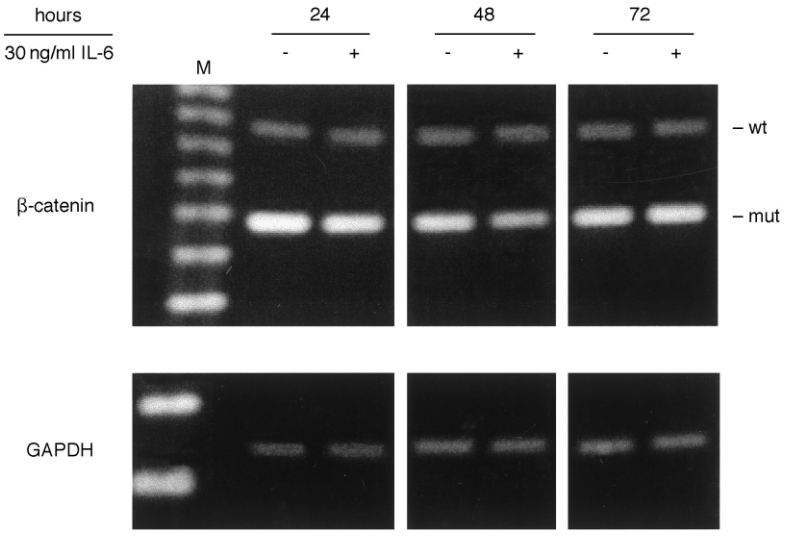

Fig. 5. Expression of β-catenin mRNA in HepG2 cells incubated without (−) or with (+) 30 ng/ml IL-6 for the indicated times. mRNA was isolated and RT-PCR was performed as previously described. RT-PCR with the GAPDH primers was performed as a control for the same amount of RNA (lower panels). Wt and mut indicate the wild-type and mutant form of β-catenin mRNA, respectively present in HepG2 cells. M = 100 bp DNA ladder. The photographs shown are representative of three independent experiments. RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

like Z-Leu-Leu-Leu-CHO (MG132) of N-acetyl-Leu-Leu-Nle-CHO (ALLN), since at the relevant concentrations and times of treatment these compounds are toxic to HepG2 cells.

Finally, there were only very slight, not significant modifications, in the levels of β-catenin mRNA in HepG2 cells after 24, 48 or 72 h of treatment with IL-6 (Fig. 5). The results shown are in the linear range of PCR amplification.

4. Discussion

HepG2 cells showed a moderate expression of IL-6Rα, both in cell surface and soluble forms, but exposed more markedly the gp130 component of the IL-6 receptor. The suggests that the availability of IL-6Rα may be a limiting factor for the responsiveness of the cells to the cytokine. In addition, we excluded the possibility that HepG2 cells can secrete or contain IL-6, which might act by an autocrine/paracrine mechanism or affect the cell responses to exogenous IL-6 [4,8]. Reportedly, exogenous IL-6 either stimulates or inhibits the production of different acute-phase proteins and factors in HepG2 cells [14,33], generally exhibiting maximal plateau effects from concentrations in the range of 1–5 ng/ml. We investigated whether IL-6 may also influence the growth of HepG2 cells, but found that it produces only minimal growth-inhibitory effects and no changes in apoptosis or cell adhesion in these quite fast growing cells. Interestingly, however, IL-6 (3 or 30 ng/ml) consistently decreased the levels of wild-type β-catenin in HepG2 cells from 48 h after the onset of treatment. This effect, which occurred in the cytoplasm, but not in the cell membranes, was selective because IL-6 did not affect mutated β-catenin or other proteins such as E-cadherin and γ-catenin. An influence on α-catenin was apparent only at the higher concentration tested. In contrast, IL-4, a non-related cytokine, did not modify the content of wild-type β-catenin.

The analysis of β-catenin mRNA in HepG2 cells indicated that the effect of IL-6 was not transcriptionally mediated. This suggests that there is some post-transcriptional mechanism by which IL-6 may reduce wild-type, but not mutated β-catenin. With regard to this, proteolytic degradation of β-catenin and, with a lesser specificity, γ-catenin may occur during apoptosis [34,35]. However, IL-6 did not induce apoptosis in the HepG2 cells. Alternatively, the cell levels of β- and γ-catenin are regulated by phosphorylation of specific serine or threonine residues localised at the N-terminus region of these proteins. The enzyme GSK3β-catalyses such phosphorylations and triggers the degradation of the cytoplasmic proteins by the ubiquitin–proteasome pathway [24–26]. It has been noted, however, that γ-catenin is less sensitive to this proteolytic regulation than β-catenin [26]. In addition, considering that the mutated β-catenin present in HepG2 cells is unable to undergo phosphorylation by GSK3β and degradation [15], we suggest that IL-6 signalling may induce by this process the selective decrease in cytoplasmic wild-type β-catenin that we observed in the cells. Consistent with this hypothesis, lithium chloride, a potent inhibitor of GSK3β activity [32], abrogated the effect of IL-6. Some recent papers have also suggested that IL-6 signalling may influence the activity of GSK3β [36,37].

While the signals that IL-6 triggers to promote cell proliferation and survival have been extensively analysed and clarified [38], it is less clear how the cytokine exerts inhibitory effects on some tumour cell types. Some studies have shown that IL-6 may impair cell adhesion and E-cadherin expression in neoplastic cells [39,40], but the influence of the cytokine on β-catenin has never been investigated. The present study suggests that the growth-regulatory effects of IL-6 may, at least in part, be related to a posttranscriptional regulation of this protein, which is able to influence cell adhesion, proliferation and survival [17–19,26]. Nevertheless, it appears that IL-6 can not counteract by this mechanism the high oncogenic levels of mutated β-catenin present in HepG2 cells or possibly in other tumour cells with similar gene mutations. We think that the possible influences of IL-6 on hepatic carcinoma, as well as on the β-catenin-related system are worthy of further investigation.

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