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Insulin-like growth factor-binding protein-2 inhibits proliferation of human embryonic kidney fibroblasts and of IGF-responsive colon carcinoma cell lines

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Abstract **So far, the physiological role of insulin-like growth factor binding protein-2 (IGFBP-2) has not been demonstrated directly. Therefore, we transfected 293 cells with an expression vector containing the CMV promoter and the complete cDNA of mouse IGFBP-2. Secretion of bioactive IGFBP-2 into conditioned medium was demonstrated by Western ligand and Western immunoblotting and quantified by specific RIA. For the analysis of cell proliferation three clones exhibiting either high or low/no IGFBP-2 expression were selected and compared to non-transfected parental 293 cells. IGFBP-2 secreting clones displayed reduced conversion of thiazolyl blue when compared to negative clones or non-transfected parental 293 cells ($P < 0.01$). The lower growth activity measured in the IGFBP-2 secreting clones was compensated in great part by the administration of exogenous IGF-I or -II. Conditioned media of IGFBP-2 secreting clones inhibited growth of IGF-responsive colon tumor cell lines (LS513, HT-29) while those of negative clones did not. In addition, conditioned medium from a clone expressing high levels of IGFBP-2 inhibited anchorage-independent growth of LS513 and HT-29 cells. In contrast, growth of an IGF-unresponsive tumor cell line (Co-115) was not affected by the conditioned media. We hypothesize that IGFBP-2 might sequester the IGFs and thus prevent them from transferring their mitogenic signals.**

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Key words: Insulin-like growth factor binding protein-2; 293 cell; Colon carcinoma cell; Cell proliferation; Growth inhibition

1. Introduction

Insulin-like growth factor binding protein-2 (IGFBP-2) is the second most abundant IGF binding protein in serum and binds IGF-II with several-fold higher affinity than IGF-I [1,2]. Increased serum levels of IGFBP-2 are found in association with fasting and a number of pathological syndromes, including non-islet cell tumor hypoglycemia (NICTH), chronic renal failure, liver cirrhosis and certain leukemias [3]. However, it is not clear whether the increase in IGFBP-2 levels plays a specific role in the pathogenesis of these diseases. Upregulation of IGFBP-2 expression after infusion of IGF [4] and in patients suffering from IGF-II secreting tumors [5] or in transgenic mice overexpressing IGF-II [6,7] suggested a positive regulation of IGFBP-2 expression by increased levels of free IGFs. IGFBP-2 might also play a major role in reproductive tissues [8–10], where its decrease was correlated with increased tumorigenicity [11].

Disruption of the IGFBP-2 gene in mice resulted in only minor phenotypic changes, suggesting functional redundancy of the IGFBPs [12,13]. However, recent findings in transgenic mice [14] and rabbits [15] overexpressing des(1–3) IGF-I or IGF-I locally in the mammary gland provided indirect evidence of an inhibitory effect of IGFBP-2 on IGF-I action. While des(1–3) IGF-I inhibited involution of the mammary gland and caused multiple pathological alterations in the transgenic mouse model [14], these changes were absent in transgenic rabbits in spite of extremely high levels of IGF-I in their milk [15]. IGFBP-2 levels in milk were markedly increased in both models; however, due to its reduced affinity for des(1–3) IGF-I, IGFBP-2 might have exerted a protective effect only in the IGF-I transgenic rabbits.

To evaluate the role of IGFBP-2 on IGF action in a defined in vitro system, we transfected 293 human embryonic kidney fibroblasts with an IGFBP-2 expression vector and analyzed cell proliferation of clones stably overexpressing IGFBP-2. The mode of IGFBP-2 action was further investigated by exposure of IGF-responsive and unresponsive colon carcinoma cell lines to media conditioned by the selected clones. We show evidence that IGFBP-2 provides negative signals in several cellular systems.

2. Materials and methods

2.1. Construction of pCMV-int-IGFBP-2

The *Eco*RI/*Not*I fragment (1450 bp) including full length mouse IGFBP-2 complementary DNA [16], kindly donated by Dr. S. Drop, Rotterdam, The Netherlands, was blunt ended and subcloned into the *Sal*I digested vector pGEM-4Z (Promega, Boehringer Ingelheim, Heidelberg, Germany). From the resulting subclone a 1480 bp restriction fragment was subcloned into *Bgl*II/*Pst*I digested mammalian expression vector pCMV-int as described previously [17]. From the resulting vector IGFBP-2 cDNA can be cut using *Eco*RI/*Pst*I digestion (Fig. 1).

2.2. Stable transfection of 293 human embryonic kidney fibroblasts

Human embryonic kidney fibroblasts (293 cells), obtained from American Type Culture Collection (ATCC, Rockville, MD, USA), were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum and subcultured every week at reduced splitting rate (1/10). 293 cells were transfected using the DMRIE-C reagent (Gibco BRL, Eggenstein, Germany). In brief, 293 cells were cultured in Petri dishes (10 cm in diameter) to 60% confluence and incubated for 12 h in 3.5 ml transfection solution containing serum-free DMEM medium, 8 μg pCMV-int-mIGFBP-2 (*Xho*I linearized) and 0.8 μg *Eco*RI linearized neomycin resistance plasmid pSV2neo (Clontech,

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Heidelberg, Germany). Monolayers were washed once in serum-free medium and kept in culture medium for 24 h. Positive clones were selected by G418 (Gibco BRL; 250 μg/ml) in the culture medium for 2 weeks. G418 was replaced every 2 days. Colonies were isolated from the Petri dish and subcultured several times. Genomic DNA from selected clones was isolated using Wizzard genomic DNA purification system (Promega). 10 μg of genomic DNA were *Eco*RI/*Pst*I digested, separated by 1% TAE agarose gel electrophoresis and blotted onto Nytran membranes (Schleicher and Schuell, Dassel, Germany) by capillary transfer. A fluorescein labeled riboprobe (riboprobe length: 1.5 kb) was synthesized using the Riboprobe Gemini Transcription System (Promega) and fluorescein-12-UTP (Boehringer Mannheim, Mannheim, Germany). The riboprobe was purified as described previously [18]. Hybridization and detection were carried out using the fluorescein Gene Images labeling system (Amersham Buchler, Braunschweig, Germany).

2.3. Analysis of mRNA expression

Cells were homogenized using a cell homogenizer (ART, Mühlheim, Germany) in 4 M guanidine thiocyanate, 25 mM sodium acetate and 0.835% mercaptoethanol for 25 s. RNA was pelleted in 5.7 M CsCl, 25 mM sodium acetate solution (20 h; 20°C; $200\,000\times g$). The RNA pellet was dissolved in DEPC-treated water and precipitated overnight at −20°C. The amount of RNA was calculated from the measured absorbance at 260 nm. The ratio of the absorbances at 260 nm and 280 nm was routinely higher than 1.7. The quality and quantity of the RNA was further examined by methylene blue staining of the Northern blots, where the bands of the 28S and 18S rRNA showed the typical ratio, clear shape and equal intensities of bands from different RNA samples.

For the analysis, 10 μg of total RNA was separated by formaldehyde agarose gel electrophoresis according to standard protocols and blotted onto nylon membranes by capillary transfer. For the hybridization a fluorescein labeled IGFBP-2 riboprobe was used as for Southern blot hybridization. The Northern blots were exposed to Biomax films (Sigma, Deisenhofen, Germany) for 2–100 min. The molecular size of the respective mRNA transcripts was estimated by comparison of their relative mobilities with an RNA size standard (Gibco BRL).

2.4. Analysis of IGFBP-2 expression

Conditioned media (described below) were analyzed by Western ligand blot analysis according to the method of Hossenlopp et al. [19] with modifications as previously described [6] to demonstrate the capacity of the secreted mIGFBP-2 to bind human IGF-II. Briefly, media were diluted 1:5 with sample buffer (50 mM Na_2HPO_4, pH 7.0; 1% (w/v) sodium dodecyl sulfate (SDS); 50% (w/v) glycerin), boiled (5 min) and electrophoresed on a 5% stacking/12% separating SDS-polyacrylamide gel using the Mini Protean II system (Bio-Rad, Munich, Germany). Separated proteins were transferred to a nitrocellulose membrane (Millipore, Eschborn, Germany). The blots were blocked with 1% fish gelatin and incubated with $[^{125}I]$IGF-II (10^6 cpm per blot). Binding proteins were visualized on Phospho-Imager Storm (Molecular Dynamics, Krefeld, Germany). All hybridization and washing steps were performed at 4°C.

IGFBP-2 in the conditioned media was identified by Western blot analysis using a cross-reacting rabbit antiserum to human IGFBP-2 (kindly provided by Dr. M. Elmlinger, Universitäts-Kinderklinik Tübingen, Germany). This antiserum has successfully been used for immunoprecipitation of mIGFBP-2 from mouse serum [18]. Membranes were prepared as described above with the single exception that the proteins were separated under reducing conditions, and incubated with human IGFBP-2 antiserum (1/1000) for 1 h and with peroxidase coupled anti-rabbit IgG antibody (Dianova, Hamburg, Germany). Signals were generated using diaminobenzidine (Sigma). IGFBP-2 levels in conditioned media were quantified by radioimmunoassay [3].

2.5. Preparation and evaluation of conditioned media

For preparation of conditioned media from selected pCMV-IGFBP-2 transfected 293 cell clones, confluent monolayers in 175 cm^2 cell culture flasks (Greiner) were washed two times with phosphate buffered saline (PBS) and incubated in 30 ml serum-free DMEM for 24 h. Conditioned media were harvested, centrifuged and stored aliquoted at −20°C until further use. The conditioned media were analyzed by use of different colon carcinoma cell lines (HT-29, LS513 and Co-115). Colon carcinoma cells were maintained in DMEM/F12 containing 5% FCS. For the proliferation assay, cells were plated into 96-multiwell plates (Greiner) at different densities (HT-29: 2×10^3 cells/well; LS513 and Co-115: 5×10^3 cells/well). Conditioned media were diluted 1:2 in the respective culture media with reduced serum concentrations (final concentration: 1% FCS). Cells were maintained in a total volume of 200 μl for 5 days.

2.6. Cell proliferation assay

Proliferation was assessed in DMEM medium containing 0.5% FCS (assay medium). Cell monolayers were washed with PBS, trypsinized (0.05% trypsin/0.02% EDTA), pelleted, resuspended in assay medium and distributed at 2×10^4 cells/well into 96-well flat-bottomed microtiter plates (Nunc, Wiesbaden-Biebrich, Germany), in the presence or absence of recombinant IGF-I, -II or Long R^3 IGF-I (Mediagnost, Tübingen, Germany) at concentrations between 0 and 300 ng/ml in a final volume of 200 μl. After five days cell proliferation was assessed, based on the conversion of thiazolyl blue (MTT) into blue formazan as described previously [20]. The measurements were carried out in triplicates. Statistical analysis was performed using Student's *t*-test.

2.7. Methylcellulose assay

Anchorage-independent growth was examined in a methylcellulose-based clonogenic assay [21]. In brief, 10^3 cells were suspended in medium containing 10% FCS, 20% conditioned media from parental 293 cells (293-0) and from the IGFBP-2 secreting cell clone 293-10 and 0.9% methylcellulose (Fluka, Deisenhofen, Germany) and plated into 35 mm bacteriological Petri dishes (Greiner). As a control serum-free medium was used instead of cell conditioned medium. Cells were incubated at 37°C and 5% CO_2 for 1 week. Colonies of more than 50 cells were counted under an inverted microscope. All samples were set up in triplicate.

3. Results

3.1. Transfected 293-cells express IGFBP-2 mRNA and produce biologically active IGFBP-2

Genomic integration of the pCMV-int-mIGFBP-2 vector was demonstrated by Southern blot hybridization in 10 of 16 clones analyzed (not shown). In the positive clones a 1.5 kb band was detected after *Eco*RI/*Pst*I digestion as expected. The endogenous IGFBP-2 gene was detected as a signal of 5 kb (not shown).

The positive clones expressed various levels of IGFBP-2 mRNA as demonstrated by Northern analysis (Fig. 2A). Strong mRNA expression was found in clones 10 and 14, intermediate expression was seen in clones 5 and 18. Low mRNA expression was found in clone 13, whereas no expression was seen in clones 4 and 11 as well as in untransfected 293 cells (0). The signal between the 1.3 kb and the 2.4 kb molecular weight standard bands corresponds to the length of the endogenous mRNA transcript from mouse liver RNA (1.7 kb).

Quantitation of IGFBP-2 levels in the media conditioned by clones 4, 13, 11, 5, 14 and 10 using a specific radioimmuno-

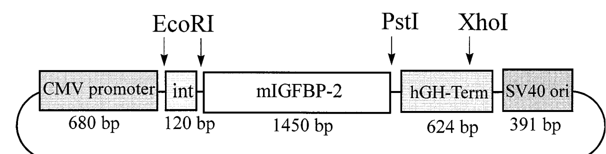

Fig. 1. Schematic representation of pCMV-int-mIGFBP-2. The construct was cloned as described in Section 2. The *Xho*I-digested construct used for the transfection includes the 680 bp CMV promoter, 120 bp rat insulin II intron A sequences, the 1450 bp mouse IGFBP-2 cDNA, and 420 bp of the terminating sequences of the human growth hormone gene.

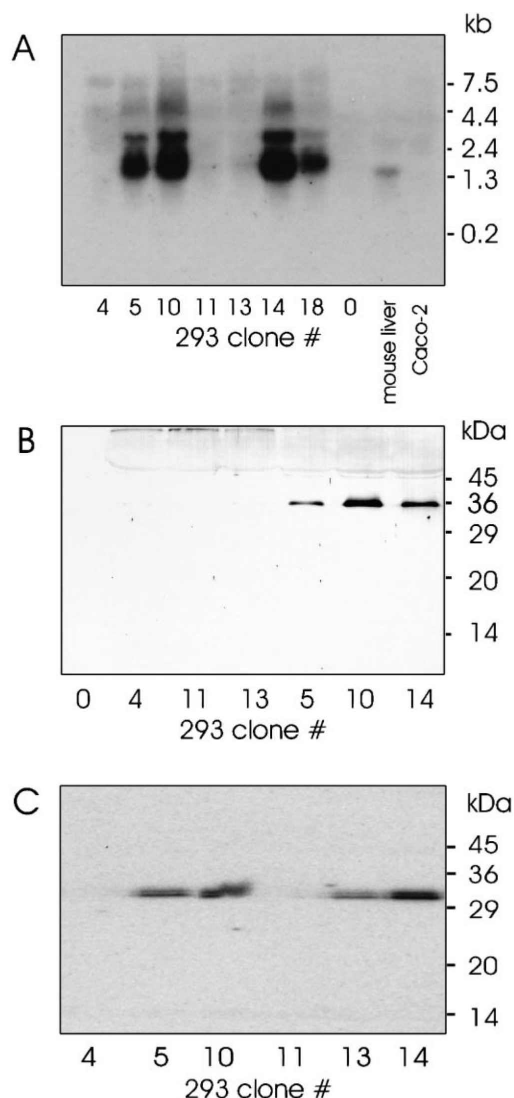

Fig. 2. A: Expression of mIGFBP-2 mRNA in selected clones of 293 cells demonstrated by Northern blot hybridization. The rate of expression exceeds the positive control (mouse liver) by severalfold. No signal was found in Caco-2 cells which are known to express only very small amounts of IGFBP-2 (exposure time: 3 min). B: Identification of IGFBP-2 in the 293 cell conditioned media by Western blot analysis. mIGFBP-2 was detected as described in Section 2 using rabbit anti-hIGFBP-2 antiserum which is known to crossreact with mouse IGFBP-2. C: Secretion of IGFBP-2 into conditioned media by selected 293 clones shown by Ligand blot analysis. Cells were washed two times and kept serum-free for 24 h. Equal amounts of cell conditioned media were analyzed as described in Section 2.

assay revealed concentrations of 0, 13, 61, 79, 180 and 489 ng/ml per day, respectively. Western blot analysis using an antiserum specific for IGFBP-2 detected a single band of the expected size (34 kDa) in conditioned media from clones 5, 10, and 14 under reducing conditions (Fig. 2B). The capacity of IGFBP-2 to bind IGF-II was shown by ligand blot analysis using [^{125}I]IGF-II (Fig. 2C). IGF-II binding was strong in media of clones 10 and 14, intermediate in those from clones 5 and 13, and low or absent in media from clones 4 and 11. Under non-reducing conditions, IGFBP-2 appeared as a double band in the range of 32 kDa.

3.2. Growth activity of selected clones

In medium containing 0.5% or 1% FCS, proliferation of 293 clones secreting high amounts of IGFBP-2 was significantly reduced ($P < 0.001$) in the absence of exogenous growth factors (Fig. 3A) when compared to 293 clones with low IGFBP-2 production or the parental cells (293-0). This reduction was completely reverted upon addition of exogenous IGF-I or IGF-II. Representative dose-response curves for two clones secreting high or low amounts of IGFBP-2 are shown in Fig. 3B. By contrast Long R^3 IGF-I, an IGF-I analogue with no affinity for the IGFBPs [22], stimulated

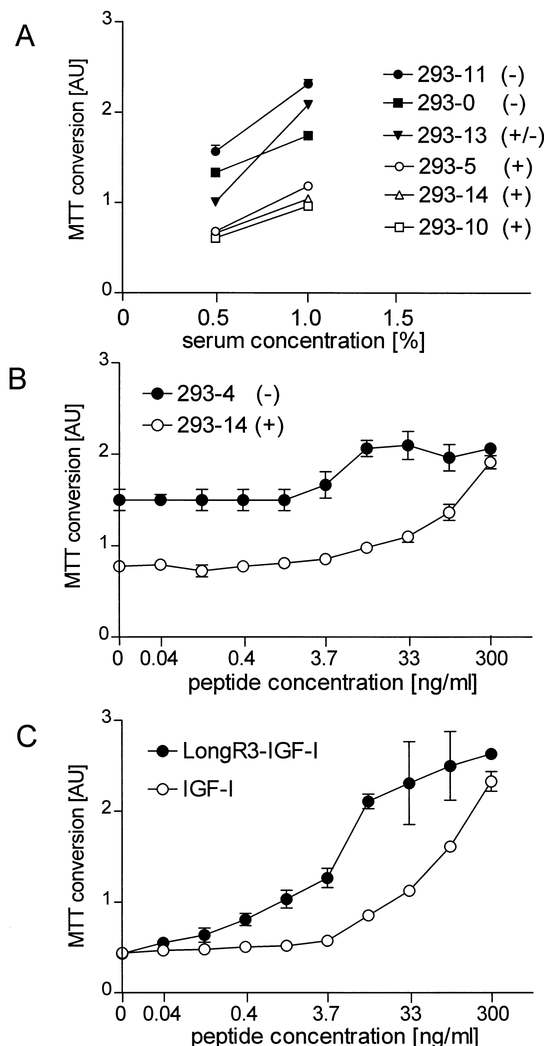

Fig. 3. A: Decreased proliferative activity in 293 clones with increased IGFBP-2 secretion. Selected 293 clones were kept in different concentrations of FCS for 5 days and analyzed for their proliferative activity as described in Section 2. Measurements were carried out in triplicate. The experiment was performed twice and a representative set of data is given. B: Decreased proliferative activity in 293-14 cells is reconstituted by the addition of exogenous IGF-I. Cells were kept in the presence or absence of IGF-I in 0.5% FCS containing culture medium for 5 days and assayed as described in Section 2. Measurements were carried out in triplicate. The experiment was performed twice and a representative set of data is given. C: Stimulation of proliferative activity in 293-10 cells using rhIGF-I and Long R^3 IGF-I. Cells were kept in the presence of various IGF-I concentrations in 0.5% FCS containing culture medium for 5 days and assayed as described in Section 2. Measurements were carried out in triplicate.

proliferative activity of IGFBP-2 secreting 293 cell clone 10 at 10-fold lower concentrations when compared to rhIGF-I (Fig. 3C). Identical results were obtained in 293-5 cells, whereas no discriminatory effect of rhIGF-I and long R3 IGF-I was observed in controls or in the parental cell line (not shown).

3.3. IGFBP-2 inhibits proliferation of IGF-responsive colon carcinoma cell lines

To demonstrate effects of media conditioned by the selected 293 clones, several colon carcinoma cell lines were exposed to these media containing IGFBP-2 at concentrations between 13 ng/ml and 489 ng/ml in the presence or absence of exogenous IGF-II (50 ng/ml). In LS513 (Fig. 4A) and HT-29 (Fig. 4B) colon carcinoma cells media conditioned by clones 10, 14 and 13 significantly reduced proliferative activity when compared to media conditioned by controls (clone 11 and 0 which represents parental 293 cells). However, when the conditioned media were supplemented with exogenous IGF-II (50 ng/ml)

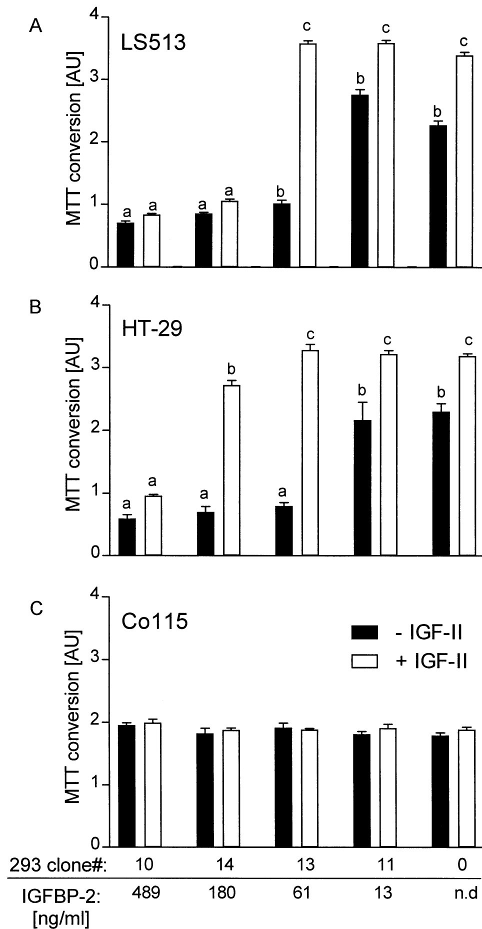

Fig. 4. Effects of media conditioned by selected 293 cell clones on proliferative activity of different colon carcinoma cell lines in the absence (filled bars) or presence (open bars) of exogenous IGF-II. Two IGF-responsive colon carcinoma cell lines (A: LS513; B: HT-29) and one IGF-unresponsive cell line (C: Co115) were analyzed. The cells were kept for 5 days before the proliferation assays were performed as described in Section 2. At the bottom of the figure the 293 clone number used for the preparation of the conditioned medium as well as the respective IGFBP-2 concentration is indicated. Different superscripts indicate significant ($P<0.01$) differences. Measurements were carried out in triplicate. The figure shows representative sets of data of at least two experiments.

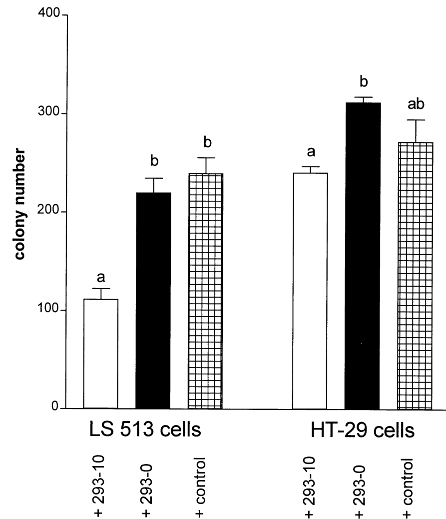

Fig. 5. mIGFBP-2 inhibits anchorage-independent growth of LS513 and HT-29 colon carcinoma cells in methylcellulose. 10^3 cells were seeded in triplicate in the presence of conditioned medium (20%) containing high (293-10) or undetectable (293-0) amounts of mIGFBP-2. As a control serum-free medium was used. Colonies of more than 50 cells were counted after 1 week. Values are means of triplicates ± S.D. and representent one of three (LS513) or two (HT-29) independent experiments. Different superscripts indicate significant differences ($P<0.01$).

the negative effects were reverted dependent on the IGFBP-2 concentration. The negative effects exerted by the different conditioned media were reverted partially for clones 10 and 14 and were reverted completely for clone 13. Moreover, IGF-II at 50 ng/ml was sufficient to significantly increase proliferation of HT-29 and LS513 cells when exposed to media conditioned by clone 11 and parental 293 cells (0). No effect of either IGFBP-2 or IGF-II was observed on growth activity of IGF-unresponsive Co-115 cells (Fig. 4C).

3.4. Anchorage-independent growth is inhibited in HT-29 and LS513 colon carcinoma cells

Anchorage-independent colony formation reflects the tumorigenic potential of the cell [23]. Therefore we investigated the effects of media conditioned by two different selected 293 cell clones containing high (293-10: 489 ng/ml) or undetectable (293 parental cells) amounts of IGFBP-2 (Fig. 5). Starting with 10^3 LS513 colon carcinoma cells after 1 week 240 ± 16 colonies were counted (24% cloning efficiency). Colony formation (112 ± 11) was inhibited by 50% when cells were coincubated in medium containing high amounts of IGFBP-2, but no significant inhibition was measured when coincubated with parental 293 cell conditioned medium (220 ± 15 colonies). Similar results were obtained in HT-29 colon carcinoma cells, which is known not to express IGFBP-2 [24]. Cloning efficiency was 27.3% when no conditioned medium was present during the assay. After coincubation in high IGFBP-2 concentrations containing media colony formation was significant decreased by 23% (241 ± 6) if compared to medium conditioned by parental 293 cells (313 ± 5). No significant difference was measured in both cell lines if medium conditioned by untransfected 293-0 cells was compared with serum-free control medium.

4. Discussion

IGFBP-2 is known to be upregulated in different pathological or unphysiological situations like trauma [25,26], certain tumors [5,27–30] or during starvation [3]. However, the function of IGFBP-2 remains unclear so far, since different in vitro studies concerning the biological effects of IGFBP-2 have led to contradictory assumptions and results. Either negative [31–34] or positive effects [35,36] have been observed upon administration of IGFBP-2 in different cell systems. The findings that retinoic acid- [37–39] or dexamethasone- [40] mediated growth inhibition is accompanied by increased IGFBP-2 expression and that specific IGFBP-2 proteolysis results in reduced affinity for IGF-II [39] suggest negative growth regulation. Furthermore, increased proliferation has been demonstrated in stably transfected IEC cells by use of an IGFBP-2 antisense construct [41]. But it also has been concluded from several in vitro studies [42–45], where IGFBP-2 expression correlated positively with a proliferative state of the cells, that IGFBP-2 might be an inducer of cell growth rather than an inhibitor. According to these studies synergistic mechanisms have been postulated between IGFBP-2 and the IGFs. In addition in osteosarcoma cells even IGF independent growth stimulation has been suggested for IGFBP-2 [36].

To determine potential effects of IGFBP-2 overproduction in a defined cell culture model we stably transfected 293 cells. The transfection resulted in the establishment of different cell clones with various levels of IGFBP-2 overexpression. To define the effect of IGFBP-2 overproduced in vitro, we analyzed the different clones and measured the effects of conditioned media derived from IGFBP-2 secreting 293 cells also in different tumor cell lines. Transfected 293 cells were identified by the presence of a restriction enzyme genomic DNA fragment (1.5 kb) in accordance with the restriction map from the expression construct. All positive clones expressed an mRNA transcript of about 1.7 kb, matching the length of the endogenous IGFBP-2 gene product from mouse liver.

Transfected 293 cells secreted various amounts of biologically active IGFBP-2 as demonstrated by Western ligand and immunoblotting. Two bands appeared under non-reducing conditions, both of which were able to bind IGF-II and both were recognized by a specific antiserum (not shown). Two bands (33 kDa and 37 kDa) representing IGFBP-2 have also been demonstrated by others [46]. Using reducing SDS-PAGE, only one band was detected, indicating the presence of two IGFBP-2 isoforms.

IGFBP-2 significantly reduced proliferation of stably transfected 293 cells. Furthermore, the negative effects were completely abolished upon the addition of exogenous IGF-I (or IGF-II; not shown) at high concentrations and Long R^3 IGF-I in lower concentrations when compared to rhIGF-I. In two IGF-responsive colon cancer cell line (HT-29 cells and LS513 cells) conditioned media from IGFBP-2 secreting 293 cells, negative effects on proliferation were confirmed and similarly compensated by the administration of exogenous IGFs. Moreover, IGFBP-2 negatively influenced the capacity of two colon carcinoma cell lines to form colonies under anchorage-independent growth conditions in methylcellulose. Most interestingly, the extent of inhibition exerted by IGFBP-2 correlates with inhibition of proliferation and colony formation by αIR3 in the two colon carcinoma cell lines [47]. This suggests that IGFBP-2 might also impair the tumorigenicity of these cells in vivo. Taken together the findings indicate that mIGFBP-2 may act by interfering with the interaction between the IGFs and their receptors and thereby prevent the transmission of their mitogenic signals. Thus, under different levels of IGFBP-2 overproduction, no IGF-independent effects could be observed. IGFBP-2 was detected on the cell surface of colorectal cancers [27], and it was further demonstrated that binding occurs to heparin and proteoglycans [48,49]. However, the biological consequences of membrane attachment remain to be elucidated. As a potential positive mechanism it might be speculated that membrane associated IGFBP-2 concentrates the IGFs in close vicinity of their receptors and thereby increases their mitogenic potential. In the different positive clones membrane association of IGFBP-2 was demonstrated in crosslinking experiments using purified membranes whereas no IGFBP-2 association was found in negative clones (data not shown). However, the fact that Long R^3 IGF-I, which does not interact with IGFBP-2 [22], was more potent in stimulating cell proliferation when compared with rhIGF-I argues strongly against IGFBP-2-mediated positive signals in our cell culture model. In addition, it has been shown that both soluble and membrane-associated IGFBP-2 competes with IGF receptors for the IGFs [33]. Neither IGFBP-2 containing conditioned media, nor the administration of IGFs were able to modulate proliferation in Co115 cells. Co115 cells are known to be IGF-unresponsive since administration of exogenous IGFs does not lead to IRS-1 phosphorylation despite IGF-I receptor expression [50].

In mice divergently selected for high or low 8-week body weight, serum levels of IGFBP-2 were also clearly increased in the low and decreased in the high weight mouse line, when compared to randomly bred mice [18], which also points to a negative effect on growth. Furthermore, increased IGFBP-2 expression was observed in the mammary glands of IGF-I transgenic rabbits [15]. Revealing IGFBP-2 as a negative growth factor in vitro, increased expression in these models might be interpreted as an antagonistic response of the IGF system to high IGF concentrations in order to prevent major pathological effects. In 293 cells IGFBP-2 regulation by IGF-I, -II and insulin [51] was demonstrated. According to the strong growth inhibitory potential of IGFBP-2, increased IGFBP-2 levels upon the addition of exogenous IGF-I, -II or insulin in 293 cells point to an antagonistic function in 293 cells. Moreover, the very common observation of increased IGFBP-2 levels in conditioned media from different tumor cell lines, which had led to the assumption that IGFBP-2 represents a positive growth factor (see above), might thus be interpreted in the same way as an autocrine response of the IGF system against high proliferation in vitro.

In summary, mIGFBP-2 displayed negative growth effects in all IGF-responsive cell lines investigated. The negative effects were abolished by the addition of exogenous IGFs. It is concluded from our data that IGFBP-2 represents an important inhibitor of cell proliferation in 293 cells and different IGF-responsive colon carcinoma cell lines.

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