Interferon α_2 recombinant and epidermal growth factor modulate proliferation and hypusine synthesis in human epidermoid cancer KB cells

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We previously found that interferon α_2 recombinant (IFNα) increases the expression of epidermal growth factor receptor (EGF-R) in the human epidermoid cancer KB cell line. Here we report the effects of IFNα and epidermal growth factor (EGF) on KB cell cycle kinetics. IFNα (1000 i.u./ml) for 48 h decreased the S-phase fraction and diminished the expression of Ki67 and proliferating cell nuclear antigen on KB cells. Incubation of IFNα-treated KB cells with 10 nM EGF for 12 h reversed these effects. We then studied several biochemical markers of cell proliferation. Ornithine decarboxylase activity was decreased to about one-tenth by IFNα and partly restored by EGF. Hypusine is contained only in eukaryotic initiation factor 5A and its levels are correlated with cell proliferation. IFNα decreased hypusine synthesis by 75%; exposure of cells to EGF for 12 h restored hypusine synthesis almost completely. We also studied the effects of IFNα on the cytotoxicity of the recombinant toxin TP40, which inhibits elongation factor 2 through EGF-R binding and internalization. IFNα greatly enhanced the TP40-induced inhibition of protein synthesis in KB cells. In conclusion, IFNα, which affects protein synthesis machinery and increases EGF-R expression, enhances the tumoricidal activity of TP40 and hence could be useful in the setting of anti-cancer therapy.

INTRODUCTION

Epidermal growth factor (EGF) activates polyamine uptake and synthesis in cancer cells and increases the expression and activity of ornithine decarboxylase (ODC) [1,2]. Polyamines have an important role in the regulation of cell growth [3] and are involved in the biosynthesis of hypusine [N^{ϵ}-(4-amino-2-hydroxybutyl)lysine], an unusual basic amino acid found only in eukaryotic translation initiation factor 5A (eIF-5A, previously designated eIF-4D) [4,5]. Hypusine is formed by the transfer of the butylamine portion of spermidine to the ϵ-amino group of a specific lysine residue of eIF-5A [6–8] and by the hydroxylation at C-2 of the incoming 4-aminobutyl moiety [9–12]. eIF-5A probably acts in the final stage of the initiation phase of protein synthesis by promoting the formation of the first peptide bond [13]. Hypusine plays a key role in the regulation of eIF-5A function because eIF-5A precursors, which do not contain hypusine, have little, if any, activity [14]. The correlation between hypusine content and cell proliferation suggests that hypusine might have a role in cell growth and differentiation [15–18]. In fact, eIF-5A and hypusine are vital for the growth of *Saccharomyces cerevisiae* [19]. Moreover, agents that decrease cell hypusine levels [20–24] inhibit the growth of mammalian cells, demonstrating that hypusine is crucial for the proliferation of eukaryotic cells [25].

We previously demonstrated that interferon α_2 recombinant (IFNα) inhibits growth, up-regulates the expression of the receptor for EGF (EGF-R) and enhances the proliferative response of human epidermoid cancer KB cells to EGF [26,27]. The latter effect is paralleled by an increased tyrosine phosphorylation of cellular proteins and of EGF-R itself [27]. Therefore IFNα increases EGF-R function because the transduction pathway of EGF-R is activated by the tyrosine phosphorylation of EGF-R and of multiple cytoplasmic proteins [28]. These results suggested that increased EGF-R expression could be a protective response of tumour cells [29]. In the present study we evaluated whether EGF could reverse IFNα-induced KB cell cycle perturbation. We also studied the effects of IFNα and EGF on ODC activity and evaluated whether the recovery of IFNα-treated KB cell proliferation induced by EGF is paralleled by the restoration of hypusine synthesis. Inhibition of protein synthesis is a new strategy in tumour cell killing; in this context the contemporaneous inhibition of initiation and elongation factors of protein synthesis is an attractive prospect. Therefore we evaluated the effects of IFNα on KB cell cytotoxicity induced by the recombinant toxin TP40, which inhibits elongation factor 2 (EF2) after binding to EGF-R [30].

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium, BSA and fetal bovine serum were purchased from Flow Laboratories (Milan, Italy). Tissue culture plasticware was from Becton Dickinson (Lincoln Park, NJ, U.S.A.). IFNα was donated by Schering-Plough (Newark, NJ, U.S.A.). Receptor grade EGF was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Anti-EGF-R monoclonal antibody (mAb) 108.1 was a gift of Dr. Joseph Schlessinger (New York University Medical Center, New York, NY, U.S.A.). [*terminal methylenes*-^{3}H]Spermidine,3HCl (15 Ci/mmol) was purchased from DuPont (Toronto, Ontario, Canada). Hypusine and deoxyhypusine standards were kindly supplied by Dr. M. H. Park (NIH, Bethesda, MD, U.S.A.). TP40

Abbreviations used: EF2, elongation factor 2; EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor; eIF-5A, eukaryotic initiation factor 5A; ID_{50}, concentration of agent able to induce 50% inhibition of protein synthesis; IFNα, interferon α_2 recombinant; mAb, monoclonal antibody; ODC, ornithine decarboxylase; PCNA, proliferating cell nuclear antigen.

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was a gift from Dr. Ira Pastan (NIH, Bethesda, MD, U.S.A.). Other reagents and chemicals were obtained from Sigma Chemical Co. All chemicals were of analytical grade.

Cell culture

The human oropharyngeal epidermoid carcinoma KB cell line, obtained from the American Type Tissue Culture Collection (Rockville, MD, U.S.A.), was grown in Dulbecco's modified Eagle's medium supplemented with heat-inactivated 10% (v/v) fetal bovine serum, 20 mM Hepes, 100 i.u./ml penicillin, 100 μg/ml streptomycin, 1% (w/v) L-glutamine and 1% (w/v) sodium pyruvate. The cells were grown in a humidified atmosphere of air/CO_2 (19:1) at 37 °C.

Western blot analysis

KB cells were seeded in 100 mm dishes and grown for 48 h with or without 1000 i.u./ml IFNα and were thereafter exposed for the indicated times to 10 nM EGF at 37 °C. For cell extract preparation, the cells were washed twice with ice-cold PBS, scraped and centrifuged for 30 min at 4 °C in 1 ml of lysis buffer [1% (v/v) Triton X-100/0.5% sodium deoxycholate/0.1 mM NaCl/1 mM EDTA (pH 7.5)/10 mM Na_2HPO_4 (pH 7.4)/10 mM PMSF/25 mM benzamidine/1 mM leupeptin/0.025 unit/ml aprotinin]. Equal amounts of cell proteins were separated by SDS/PAGE. The proteins on the gels were electrotransferred to nitrocellulose and reacted with anti-EGF-R 108.1 mAb [27].

FACS analysis of Ki67 and proliferating cell nuclear antigen (PCNA)

KB cells were treated with IFNα and/or EGF as described above and then harvested in EDTA solution (0.1% EDTA in PBS without Ca^{2+}/Mg^{2+}, pH 7.4). Cells (2×10^6) were centrifuged and resuspended in washing medium [PBS/1% human serum/0.1% NaN_3 (pH 7.4)]. Appropriately diluted anti-Ki67 or anti-PCNA mAbs (Dako, Bastrup, Denmark) were added to the pellet; an irrelevant IgG_1 mAb was used as control. After 30 min at 4 °C, KB cells were washed twice in washing medium and cell pellets were incubated with FITC-labelled goat anti-mouse Ig (Technogenetics, Milan, Italy) diluted 1:20 at 4 °C for 30 min in the dark. After two washings with PBS, KB cells were analysed by a FACScan flow cytometer (Beckton Dickinson). Data analysis was performed with the CONSORT 30 computer program; live cell gating was determined by light-scattering parameters [forward scatter (FSC)-side scatter (SSC)]. The data were acquired after analysis of at least 20000 events from three different experiments. Statistical evaluation was performed with the BMDP statistical software package.

FACS analysis of cell cycle distribution

KB cells were treated with IFNα and/or EGF as described above and with trypsin, washed twice with PBS without Ca^{2+}/Mg^{2+} and fixed in 70% (v/v) ethanol. 10^6 KB cells were incubated at room temperature for 30 min in 1 ml of a propidium iodide staining solution (50 μg/ml in PBS without Ca^{2+}/Mg^{2+}, pH 7.4). DNA analysis was performed in duplicate with a FACScan flow cytometer linked to a Hewlett-Packard computer. Cell cycle data analysis was performed with the CELL-FIT program (Becton Dickinson). Pulse area–pulse width gating was performed to avoid doublets from the G_2/M region. The percentage proliferation index was determined as the ratio of the sum of cell percentages in G_1, G_2, S and M phases to the sum of cell percentages in G_0, G_1, G_2, S and M phases of the cell cycle. The evaluation of the cell percentage in the G_1 phase was obtained by double labelling of cells with propidium iodide and anti-Ki67 FITC mAb. Then G_1–G_0 cells were gated and Ki67-expressing cells were considered to be in the G_1 phase. The data were acquired after analysis of at least 20000 events from three different experiments. The statistical significance of the difference in cell cycle pattern with and without the addition of IFNα and EGF was evaluated by analysis of variance. Statistical evaluation was performed with the BMDP statistical software package.

Protein synthesis inhibition assay

KB cells were seeded at 2.5×10^5 per well in 24-well plates. After 24 h of incubation at 37 °C, KB cells were grown with or without 1000 i.u./ml IFNα for 48 h. Different concentrations of TP40, diluted with 0.2% (w/v) human serum albumin, were added to the cells. Then KB cells were incubated at 4 °C for 60 min to allow the binding of TP40 to the cells. KB cells were washed three times with PBS/0.1% BSA and incubated at 37 °C for 5 h in complete medium as described in the Cell culture section. The medium was withdrawn and complete medium containing L-[^{3}H]leucine (45–70 Ci/mmol; Amersham, Arlington Heights, IL, U.S.A.) was added to KB cells, which were then incubated at 37 °C for 60 min. After incubation the cells were washed three times with PBS without Ca^{2+} or Mg^{2+} and dissolved in 1 ml of 0.1 M NaOH. After 5 min of incubation at 37 °C, NaOH was added to the centrifuge tubes and the proteins were precipitated in ice-cold 12% (w/v) trichloroacetic acid for 60 min. The precipitate was washed twice in 6% trichloroacetic acid and digested in 1.5 ml of 0.1 M NaOH at 56 °C for 30 min. Aliquots were assayed with the Lowry method for protein quantification and counted in scintillation liquid in a β-counter (Beckmann) to detect the incorporation of L-[^{3}H]leucine. The results are expressed as a percentage inhibition of protein synthesis as previously described [30].

Isolation, purification and identification of hypusine

KB cells were seeded in 100 mm dishes and treated as described above; 24 h before processing, 8 μl of [*terminal methylenes*-^{3}H]spermidine,3HCl (15 Ci/mmol) was added to each dish. Cell lysates were prepared with cells from 10 dishes (0.1 ml of washed cells) by suspending the cells in 4 ml of PBS, sonicating them (10 s at 70 W), and finally centrifuging them for 30 min at 25000 ***g***. The lysates were treated with solid $(NH_4)_2SO_4$ (40–80% saturation cut) and the precipitate was hydrolysed in 6 M HCl at 110 °C for 18 h. The hydrolysates were applied to 0.5 cm × 4 cm columns of AG 50 × 2 (H^+ form, 200–400 mesh) and eluted with 30 ml of 1 M HCl, 20 ml of 3 M HCl and 30 ml of 6 M HCl. The hypusine contained in the 3 M HCl fraction was determined by using a reverse-phase HPLC method described elsewhere [9,31].

ODC assay

ODC activity was determined by measuring the release of $^{14}CO_2$ from [^{14}C]ornithine. Briefly, the reaction mixture contained 100 mM glycylglycine buffer, pH 7.2, 0.2 mM pyridoxal phosphate, 5 mM dithiothreitol, 1 mM L-ornithine, 1 μCi of D,L-[1-^{14}C]ornithine and an aliquot of cell lysate (approx. 1.5 mg of protein) in a final volume of 2 ml. The reaction was accomplished in a shaking water bath for 60 min at 37 °C in glass flasks closed with an airtight rubber cap and equipped with an internal reservoir containing 0.5 ml of hyamine (Fluka, Buchs, Switzerland). The reaction was stopped by injecting 1.5 ml of 20% trichloroacetic acid. The hyamine solution was transferred

to a scintillation vial and assayed for radioactivity. One unit of ODC is defined as the amount of enzyme catalysing the release of 1 pmol of CO_2/h from L-ornithine.

RESULTS

Effects of IFNα and EGF on cell cycle kinetics and EGF-R expression in KB cells

Exposure of KB cells to 1000 i.u./ml IFNα for 48 h induced a significant decrease in the S-phase cell fraction ($P = 0.001$; Student's t test) and proliferative index ($P = 0.002$) and in the expression of both Ki67 ($P < 0.0001$) and PCNA ($P < 0.0001$) as evaluated with a cytofluorimetric assay. EGF (10 nM) did not cause significant changes in the S-phase cell fraction or in the other cell proliferation markers (see Tables 1 and 2). Incubation of IFNα-treated KB cells with 10 nM EGF for 12 h restored the S-phase fraction, proliferative index and expression of Ki67 and PCNA to almost control values (Tables 1 and 2). Exposure of KB cells to 1000 i.u./ml IFNα for 48 h resulted in an almost doubling of EGF-R level. Exposure of KB cells to 10 nM EGF for 10 min or for 12 h did not affect EGF-R levels, as demonstrated by Western blot assay (Figure 1). Therefore the expression of EGF-R was increased by IFNα but not by EGF.

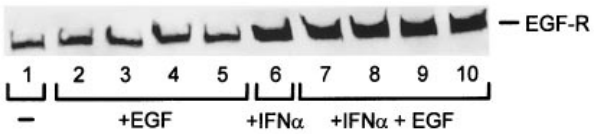

Figure 1 Effects of IFNα and EGF on EGF-R expression in KB cells

Cell proteins (150 μg per lane) were assessed by Western blot analysis for EGF-R (see the Materials and methods section). Cells were cultured for 48 h without (lanes 1–5) or with (lanes 6–10) 1000 i.u./ml IFNα and incubated for various times with 10 nM EGF. Lane 1, untreated controls; lane 2, 10 min EGF; lane 3, 20 min EGF; lane 4, 6 h EGF; lane 5, 12 h EGF; lane 6, IFNα; lane 7, IFNα and 10 min EGF; lane 8, IFNα and 20 min EGF; lane 9, IFNα and 6 h EGF; lane 10, IFNα and 12 h EGF. The experiment shown is one of three experiments that gave similar results.

Table 1 Effects of IFNα and EGF on KB cell cycle distribution

The percentage distribution of KB cells in the cell cycle was evaluated by FACS analysis after nuclear labelling with propidium iodide. The cell fraction in the G_1 phase was calculated by gating KB cells that expressed Ki67 antigen, whereas G_0 values were derived by subtracting G_1 from the G_0–G_1 fraction. The results are expressed as means ± S.E.M. for the data acquired after the analysis of at least 20000 events from three different experiments performed in triplicate.

	Proportion of cells (%)			
Treatment	G_0 phase	G_1 phase	S phase	G_2–M phase
None	10.00 ± 4.40	52.00 ± 2.15	26.00 ± 0.87	12.00 ± 1.38
EGF 10 min	9.00 ± 2.32	51.30 ± 0.88	25.80 ± 0.52	13.90 ± 0.92
EGF 20 min	8.00 ± 1.15	50.80 ± 0.36	27.00 ± 0.50	14.20 ± 0.29
EGF 6 h	18.00 ± 4.32	38.60 ± 1.60	28.50 ± 1.01	14.90 ± 1.71
EGF 12 h	19.00 ± 4.01	37.60 ± 1.32	28.50 ± 1.20	14.90 ± 1.49
IFNα	42.00 ± 1.44	33.70 ± 0.24	16.00 ± 0.74	8.30 ± 0.46
IFNα + EGF 10 min	40.00 ± 1.73	34.00 ± 0.52	17.20 ± 0.72	8.80 ± 0.49
IFNα + EGF 20 min	38.00 ± 2.31	28.60 ± 0.80	23.90 ± 0.77	9.50 ± 0.74
IFNα + EGF 6 h	29.00 ± 2.58	35.20 ± 0.76	25.00 ± 0.83	10.80 ± 0.99
IFNα + EGF 12 h	18.00 ± 4.34	44.00 ± 1.96	26.30 ± 0.82	11.70 ± 1.56

Table 2 Effects of IFNα and EGF on PCNA and Ki67 expression and on the percentage proliferation index (PI) of KB cells

The cells were labelled with anti-Ki67 or anti-PCNA mAbs and, after incubation with an FITC-conjugated anti-mouse antibody, they were analysed by FACS. The levels of Ki67 and PCNA are expressed as mean equivalent standard fluorescence calculated by comparing the fluorescence intensity of the antigen with that of a standard fluorescent microsphere. The percentage PI was calculated as described in the Materials and methods section. The results are the means ± S.D. (PCNA, Ki67) or means ± S.E.M. (PI) for data acquired after the analysis of at least 20000 events from three different experiments performed in triplicate.

	Mean equivalent standard fluorescence		
Treatment	PCNA	Ki67	PI (%)
None	14500 ± 500	15000 ± 510	90.00 ± 4.40
EGF 10 min	14000 ± 200	15000 ± 708	91.00 ± 2.32
EGF 20 min	14000 ± 211	16000 ± 616	92.00 ± 1.15
EGF 6 h	14000 ± 401	14000 ± 408	82.00 ± 4,32
EGF 12 h	13500 ± 421	13500 ± 680	81.00 ± 4.01
IFNα	8000 ± 306	7000 ± 200	58.00 ± 1.44
IFNα + EGF 10 min	8000 ± 515	7500 ± 210	60.00 ± 1.73
IFNα + EGF 20 min	9000 ± 220	8500 ± 405	62.00 ± 2.31
IFNα + EGF 6 h	10000 ± 341	10000 ± 415	71.00 ± 2.58
IFNα + EGF 12 h	12500 ± 570	12500 ± 500	82.00 ± 4.34

Table 3 Effects of IFNα and EGF on ODC activity in KB cells

The ODC activity was determined by measuring the release of $^{14}CO_2$ from [^{14}C]ornithine as described in the Materials and methods section. The results are means ± S.D. for three different experiments performed in triplicate.

Treatment	ODC activity (pmol/h per mg of protein)
None (control)	75.0 ± 6.0
10 nM EGF 10 min	68.0 ± 4.2
10 nM EGF 12 h	102.0 ± 12.5
1000 i.u./ml IFNα 48 h	8.2 ± 1.5
1000 i.u./ml IFNα 48 h/10 nM EGF 10 min	12.5 ± 1.8
1000 i.u./ml IFNα 48 h/10 nM EGF 12 h	25.6 ± 2.8

Effects of IFNα and EGF on ODC activity in KB cells

ODC activity is correlated with cell cycle progression of tumour cells [1,2]. Therefore we evaluated the effects of IFNα and EGF on ODC activity in KB cells. Incubation of KB cells with 10 nM EGF for 12 h induced a 40% increase in ODC activity, whereas treatment for 48 h with 1000 i.u./ml IFNα caused a decrease in activity to one-ninth (Table 3). Exposure of IFNα-treated KB cells to 10 nM EGF for 12 h caused a 3-fold increase in ODC (Table 3) activity. The cell cycle modifications induced by IFNα and EGF were therefore associated with changes in ODC activity.

Effects of IFNα and EGF on hypusine synthesis in KB cells

Hypusine levels are correlated with cell proliferation [9,15–18,25]. Because IFNα and EGF alter the proliferative status of tumour cells we evaluated their effects on hypusine synthesis after labelling KB cells with [^{3}H]putrescine. Exposure of KB cells to 10 nM EGF for 10 min or 12 h did not significantly affect hypusine synthesis (Table 4). However, IFNα caused hypusine synthesis to decrease by approx. 75%; hypusine formation progressively returned almost to control values when the cells were incubated with EGF for 12 h (Table 4). Therefore the antiproliferative effects induced by IFNα were associated with

Table 4 Effects of IFNα and EGF on hypusine synthesis in KB cells

Hypusine levels were determined after labelling of KB cells with [*terminal methylenes*-^{3}H]spermidine,3HCl and subsequent HPLC analysis of hydrolysed cell protein extract as described in the Materials and methods section. The results are expressed as the means ± S.D. for three different experiments performed in triplicate.

Treatment	Hypusine synthesis (pmol/mg of protein)
None (control)	2.61 ± 0.20
10 nM EGF 10 min	2.52 ± 0.15
10 nM EGF 12 h	2.32 ± 0.17
1000 i.u./ml IFNα 48 h	0.74 ± 0.06
1000 i.u./ml IFNα 48 h/10 nM EGF 10 min	1.42 ± 0.09
1000 i.u./ml IFNα 48 h/10 nM EGF 12 h	2.01 ± 0.17

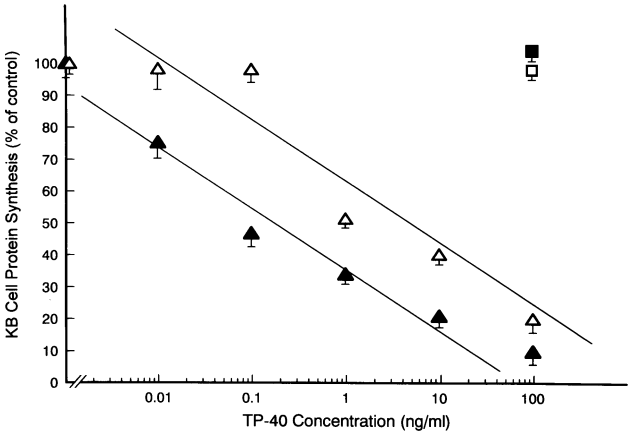

Figure 2 Effects of IFNα on cytotoxicity induced by TP40 in KB cells

KB cells were grown without (△) or with (▲) 1000 IU/ml IFNα for 48 h; control cells (□) or IFNα-treated cells (■) were incubated with 100 ng/ml TP40 and 100 nM EGF. IFNα-treated and untreated cells were incubated with various concentrations of TP40 at 4 °C for 60 min. After washes with PBS/0.1% BSA, cells were incubated at 37 °C for 5 h in complete medium; the cells were then labelled with L-[^{3}H]leucine for 60 min at 37 °C. After washes with PBS, cells were dissolved in 1 ml of 0.1 M NaOH. The proteins were precipitated in ice-cold 12% trichloroacetic acid, washed twice in 6% trichloroacetic acid and digested in 1.5 ml of 0.1 M NaOH at 56 °C for 30 min. Aliquots were counted in scintillation liquid in a β-counter. The inhibition of protein synthesis was calculated by dividing the result for each experimental point by the protein synthesis of KB cells not incubated with TP40. Points show averages of results from three different experiments performed in triplicate; bars show S.E.M.

decreased hypusine synthesis, which in turn was restored by the addition of EGF to IFNα-treated KB cells.

Effects of IFNα on cytotoxicity of TP40

We studied the effects of IFNα on the cytotoxicity of the fusion protein TP40, which binds EGF-R and inhibits EF2 through internalization mediated by EGF-R [30].The concentration of TP40 that inhibited protein synthesis by 50% (ID_{50}) in untreated KB cells was 4.9 ng/ml as evaluated with an L-[^{3}H]leucine incorporation assay (Figure 2). When KB cells were exposed to 1000 i.u./ml IFNα the ID_{50} of TP40 was 0.18 ng/ml. Moreover the effects of TP40 were antagonized by the addition of saturating concentrations of EGF. This observation demonstrates that cytotoxicity is mediated by specific binding of the fusion protein to EGF-R (Figure 2). Therefore IFNα increases the cytotoxicity of TP40 in KB cells.

DISCUSSION

Human carcinoma cells that have been exposed to cytostatic concentrations of IFNα show increased EGF-R expression at the tumour cell surface, which leads to signalling activation and subsequently to a proliferative response [27]. Here we report that IFNα decreases the proliferative index and the S-phase fraction on human epidermoid cancer KB cells. EGF reversed the proliferative status induced by IFNα. These effects were paralleled by modulation of hypusine synthesis. We also confirm that modifications of the cell cycle and hypusine synthesis induced by EGF and IFNα are paralleled by changes in ODC activity [1,2,32,33].

Agents that suppress hypusine synthesis induce reversible arrest at the G_1/S boundary of the cell cycle (34–38). In fact, D,L-α-difluoromethylornithine depresses the formation of spermidine and in consequence that of hypusine, producing a G_1/S block in 9L brain tumour cells [34]. In hydralazine-treated cloned mouse T-cells, growth arrest in late G_1 and inhibition of deoxyhypusyl hydroxylation occur concomitantly [35]. In Chinese hamster ovary cells, 2-(4-hydroxytoluene-3-yl)-4,5-dihydro-5-carboxythiazole causes both inhibition of hypusine biosynthesis and reversible cell cycle arrest in late G_1 and suppresses the proliferation of human T lymphocytes *in vitro* at the G_1/S boundary [36]. Hypusine synthesis increases after mitogen treatment of human peripheral blood lymphocytes [37]. Moreover, both polyamine [38] and hypusine levels (together with the enzymes that regulate their metabolism) [9,11,15,39,40] are correlated with normal and malignant growth. This coincides with our finding that modifications of the cell cycle induced by IFNα are paralleled by a decrease in hypusine synthesis.

The results of this study might have therapeutic implications because manipulation of protein synthesis machinery is a key feature of strategies designed to inhibit tumour cell growth. Attempts are being made to construct agents that induce tumour cytotoxicity by inhibiting the translational process. A case in point is the fusion protein TP40, which is a genetically engineered construct consisting of a recombinant toxin derived from *Pseudomonas aeruginosa* and of the targeting component, transforming growth factor α, one of the natural ligands of EGF-R, which, in turn, is overexpressed on tumour cells and is considered a tumour-associated antigen [29,30,41]. Thus TP40 preferentially recognizes cancer cells through EGF-R binding. The cytotoxic action of the targeted toxin is exerted through the physiological internalization process of the bound EGF-R [29]. After internalization, the recombinant toxin inhibits protein synthesis through ADP-ribosylation of the EF2. Moreover the density of the tumour-associated antigen, EGF-R in the present instance, on the tumour cell surface is an important limiting factor of targeting strategies [29,30,42]. Because IFNα up-regulates EGF-R expression on the cell surface and decreases hypusine synthesis, which is necessary for eIF-5A activation, we asked whether IFNα could increase the cytotoxicity of TP40, which targets EGF-R and inhibits another step of protein synthesis. We found that IFNα increased by approx. 27-fold the effect of TP40 on KB cells. This effect could be attributed both to IFNα-induced EGF-R up-regulation and to IFNα- and TP40-induced inhibition of multiple steps of protein synthesis.

In conclusion, this is the first report to correlate the inhibition of tumour cell growth induced by IFNα and the ability of EGF to antagonize the effects of IFNα by modulating hypusine synthesis. Moreover, the concurrent inhibition of eIF-5A induced by IFNα and of EF2 induced by TP40 could be relevant in the setting of anti-cancer therapy.

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