Pergamon

European Journal of Cancer, Vol. 34, No. 13, pp. 2046–2052, 1998

Printed in Great Britain
0959-8049/98/$—see front matter

PII: S0959-8049(98)00292-5

Original Paper

Expression of p68 Protein Kinase and Its Prognostic Significance During IFN-α Therapy in Patients with Carcinoid Tumours

Y. Zhou,[1] A. Gobl,[1] S. Wang,[1] M.B. Jacobsen,[2] E.T. Janson,[1] G.K. Haines III,[3] J.A. Radosevich[3] and K. Oberg[1]

[1]Endocrine Oncology Unit of Internal Medicine, Uppsala University Hospital, S-751 85, Uppsala, Sweden; [2]Department of Internal Medicine, Oslo University Hospital, Oslo, Norway; and [3]Department of Veterans Affairs, Northwestern University Medical School, Chicago, Illinois, U.S.A.

The aim of this study was to evaluate the antiproliferative effects of interferon alpha (IFN-α) on neuroendocrine differentiated cell lines and, retrospectively, to assess the prognostic significance of p68 protein kinase (PKR) induction in neuroendocrine gut and pancreatic tumour patients. Archive specimens from 56 patients were studied, 43 before IFN-α and 56 during therapy. The tissues were immunostained for p68 protein kinase (PKR) using the monoclonal antibody (MAb) TJ4C4. A significant increase in immunostaining after treatment with IFN-α compared with before treatment (3.47 ± 0.12 versus 2.72 ± 0.15, $P < 0.001$) was noted. The p68 score was significantly increased after treatment only in patients with stable disease before = 2.71 ± 0.19, after = 3.40 ± 0.14 ($P < 0.001$) or an objective response before 3.13 ± 0.22, after = 4.00 ± 0.24 ($P < 0.05$) but not in those with progressive disease (before = 2.32 ± 0.24, after 2.86 ± 0.26, NS). A low p68 score (< 3.0) during treatment was a predictor of shorter duration of response and overall survival ($P = 0.0062$ and $P < 0.0001$, respectively). Furthermore, IFN-α showed a significant antiproliferative effect (by [^{3}H]thymidine incorporation) on two carcinoid tumour cell lines in a dose-dependent manner which correlated with a dose-dependent induction of p68 mRNA and protein expression (by Northern and Western blot analysis). We conclude that IFN-α can effectively inhibit the *in vitro* growth of carcinoid tumor cell lines and upregulates the expression of p68 at both mRNA and protein levels in carcinoid tumours. The induction of p68 could be a prognostic indicator of response in patients with carcinoid tumours during IFN-α treatment. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: interferon alpha, carcinoid, neuroendocrione tumours, proliferation, p68, protein kinase, PKR, prognostic factor
Eur J Cancer, Vol. 34, No. 13, pp. 2046–2052, 1998

INTRODUCTION

INTERFERONS (IFNs) BELONG to a group of regulatory proteins which exert their antiviral, antitumour and immunomodulatory effects by binding to specific cell surface receptors by inducing the transcription of multiple genes [1–4]. Some of these genes, such as *Mx-A* and 2′,5′-oligoadenylate synthetase (*2-5OAS*), are believed to correlate with antiviral activity [5–7]. The expression of both *2-5OAS* and *p68* kinase gene products correlate with the inhibition of protein synthesis and stop the cell cycle, thereby inducing both antiviral and antitumour effects [4, 7–11].

Several studies have indicated an important role of p68 kinase (PKR) in the antitumour effects of IFN-α [4, 12–14]. In addition, the expression of p68 has been shown to correlate with the degree of cell differentiation in a variety of carcinomas [15–19]. Malignant embryonal carcinoma stem cells do not express p68 kinase after treatment with IFN-α, although *2-5OAS* can be induced [2, 20, 21]. In NIH 3T3 cells, clones expressing mutant p68 expressed a malignant phenotype with tumorigenic potential, whilst clones expressing wild-type p68 kinase did not have a malignant phenotype

Correspondence to K. Oberg.
Received 20 Mar. 1998; revised 2 Jul. 1998; accepted 21 Jul. 1998.

[12, 21]. Cos-1 cells which expressed mutant p68 had 35–70-fold higher level of mutant p68 than the wild-type p68 [14]. Furthermore, expression of wild-type p68 kinase in Hela cells resulted in apoptosis [13]. These studies suggest that p68 kinase may play a key role in cellular growth regulation and thereby exhibit antitumour actions induced by IFN-α. Very limited data have been generated regarding the growth regulation of carcinoid tumour cells and possible intracellular effects of treatment with IFN-α [22–26]. We and others have demonstrated the antitumour effects of IFN-α in patients with carcinoid tumours and its effects on tumour cell proliferation [26–32]. In the present study, we investigated the induction of p68 kinase mRNA and protein levels in neuroendocrine differentiated cell lines (Bon1 and LCC18). The induction has been correlated to cell growth inhibition in these cell lines. Furthermore, the expression of p68 kinase at the protein level has been investigated in a large cohort of patients with carcinoid tumours and correlated to antitumour response and overall survival after treatment with IFN-α.

MATERIALS AND METHODS

Cell culture

Bon1 cells derived from a lymph node metastasis of a human pancreatic carcinoid tumour [33] were cultured in a 50/50 mixture of Hams F 12K (F-12K) nutrient medium and Dulbecco's Modified Eagle's Medium (DMEM) (F-12 K/DMEM) supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/ml) and streptomycin (100 units/ml) in an atmosphere of 95% humid air, 5% CO_2 at 37°C.

LCC18 cell lines derived from a human neuroendocrine colonic tumour [34] were grown in RPMI 1640 nutrient medium supplemented with 10% (v/v) fetal bovine serum, 10^8 mol/l hydrocortisone, 5 μg/ml bovine insulin, 10 μg/ml human transferrin, 10^8 mol/l β-oestradiol, 3×10^8 mol/l selenium, penicillin (100 units/ml) and streptomycin (100 units/ml) in an atmosphere of 95% humid air, 5% CO_2 at 37°C.

IFN-α

Human recombinant IFN-α 2b was used in this study (Schering-Plough Corp., New Jersey, U.S.A.) with a specific activity of 3.0 million international units (IU) per millilitre.

P68 kinase mRNA expression

Cells were plated and allowed to grow to 80% confluence. The appropriate amount of IFN-α was added. After various incubation periods the cells were pelleted in 50-ml polypropylene tubes (Falcon, Becton Dickinson Labware, Lincoln Park, New Jersey, U.S.A.), and total RNA was isolated by using the guanidinium-phenol-chloroform method [35]. RNA (approximately 40 μg) was electrophoresed in 1% agarose gels containing formaldehyde and then transferred to a Hybond-N (Amersham, Arlington Heights, Illinois, U.S.A.) membrane for Northern blot analysis. A 1.8-kb Hind III + Pst I p68 cDNA fragment was used as probe [36]. To check loading of RNA a 1.0-kb Hind III + Xba I cDNA probe was used for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The probes were labelled by random priming using T7 Quickprime Kit (Pharmacia, Uppsala, Sweden) and [α-^{32}P]dCTP (3,000 Ci/mmol, Amersham, U.K.). Membranes were prehybridised for 1–2 h at 42°C in hybridisation solution containing 5×SSPE, 5×Denhardt's, 50% formamide, 0.5% SDS. Probe was added and hybridisation was performed for 12–16 h at 42°C. The membranes were washed once for 15 min in 2×SSC, 0.5% SDS at room temperature and once for 15 min at 30°C in the same washing solution; and then washed at 42°C with 0.1×SSC for 10 min. The membranes were exposed using storage phosphor screens (Molecular Dynamics, California, U.S.A.) for 12–16 h. Signal intensity was quantitated by scanning the target bands. The membranes were then stripped of the probe by boiling in 0.1% (w/v) SDS, cooled to room temperature, and rehybridised with a GAPDH probe. Densitometric values are expressed as a ratio of p68/GAPDH before and after treatment with IFN-α, with control values at 1 (to measure increased fold after inducing with IFN-α) or arbitrary densitometric units with GAPDH densitometry values at 1 (for statistical analysis).

Western blot analysis

10 μg of the total protein (quantified using protein assay kit, Pierce, Novakemi AB, U.S.A.) was loaded and separated in 4–15% SDS-polyacrylamide gradient gels, transferred to nitrocellulose membranes (Bio-Rad, California, U.S.A.) and saturated with 5% non-fat milk overnight at 4°C. The membranes were incubated with anti-p68 monoclonal antibody (MAb) (at 1:100 dilution) for 1 h at room temperature. After briefly rinsing twice and washing twice for 10 min with PBS containing 0.1% Tween 20, the filters were incubated for 1 h with a 1/1000 dilution of sheep antimouse IgG conjugated to horseradish peroxidase at room temperature. Finally, the filters were washed as above and developed using the ECL Western blotting kit (Amersham, U.K.).

[^{3}H]*Thymidine incorporation and cell proliferation assays*

Bon1 cells were seeded at approximately 5×10^5 cells/500 μl/well in 48-well microtitre culture plates in DMEM/F-12 K media supplemented with 10% fetal bovine serum. Cells were allowed to grow to approximately two-thirds confluence. Human recombinant IFN-α (0, 10, 100, 1,000, 10 000 IU/ml respectively) was added and cells were incubated at 37°C for 24 h. During the last 6–7 h of culture, 0.2 μCi of [^{3}H]thymidine (5.0 mCi/mmol, Amersham, U.K.) diluted with culture medium was added to each well. The culture medium was then removed.

The LCC18 cell line was seeded at 2×10^5 cells/500 μl/well in 48-well microtitre culture plates in culture medium together with human recombinant IFN-α (0, 10, 100, 1,000, 10 000 IU/ml respectively). Cells were incubated at 37°C for 24 h. During the last 6–7 h, 0.2 μCi of [^{3}H]thymidine diluted with culture medium was added to each well. The culture medium was then removed and the cells were precipitated with 10% (w/v) TCA for 10 min at 4°C.

The cells were washed three times with 1×PBS. Then 1 ml of lysate buffer (0.3 M NaOH, 0.1% SDS) was added to each well and incubated for 20 min at room temperature. The cell lysate was collected and transferred to a plastic tube and 3 ml of scintillation fluid were added to each tube. Incorporated radioactivity was determined using a beta counter (Rackbeta II Liquid Scintillation Counter 1215, LKB Wallac, Finland).

Patients' data

56 patients were retrospectively selected with histologically proven carcinoid tumours subsequently referred to the University Hospital of Oslo, Norway and Uppsala, Sweden between 1987 and 1994. These patients all had metastatic disease with no prior treatment. Biopsy specimens were taken prior to and

after various periods of IFN-α treatment (IntronA 5–9 mil IU, III-V/w.s.c.). The specimens were formalin fixed and paraffin embedded. The patients were followed up to December of 1995. The overall survival was determined from the date of initiation of treatment with IFN-α to the date of death or last follow-up of the patients. The sampling of patient specimens followed the procedure of the hospital's Ethical Committee and the study was approved by the local Ethical Committee.

Response criteria

The response criteria of treatment of the patients have been previously described [37]. Briefly, a response was defined as a > 50% decrease in the principal biochemical marker tested (U-5HIAA) and/or reduction of tumour size by > 50% on computer tomography or ultrasound. Tumour progression was defined as an increase of > 25% of the biochemical marker or tumour size. Stable disease was defined in between these boundaries. The duration of response was determined from the date of the initiation of treatment with IFN-α to the date of progression.

Immunohistochemistry

Immunohistochemistry was performed on 5 μm sections using a standard avidin-biotin complex (ABC) method. Briefly, sections were deparaffinised in xylene and rehydrated in graded ethanols. Endogenous peroxidase activity was blocked with phosphate-buffered saline (PBS) containing 0.3% hydrogen peroxide for 30 min. Nonspecific binding was blocked by incubation in 10% normal horse serum (Vector Laboratories, Burlingame, California, U.S.A.) for 45 min. The sections were then incubated overnight in a humid chamber at 4°C with the mouse Mab (TJ4C4) directed against human p68 kinase. After washing with PBS, the sections were incubated with biotinylated horse antimouse secondary antibody (1:200 in PBS, Vector Laboratories for 40 min, followed by a 45 min incubation with the ABC-Elite avidin reagent (Vector Laboratories) for 45 min, with thorough washes between steps. The antigen-antibody reaction was visualised with 3-amino-9-ethylcarbazole/H_2O_2. Sections were counterstained with haematoxylin, dehydrated and coverslipped. Paraffin sections of normal skin were used as positive controls for p68 kinase immunoreactivity. Negative controls consisted of the same tissue sections using culture medium but no p68 MAb. The specimens before and after treatment with IFN-α were included at the same batch to ensure the standardisation of staining intensity. The samples were examined blindly.

Grading of p68 kinase immunoreactivity

All immunostained sections were examined with a standard light microscope (×50) for intensity and extent of staining. The scoring procedure was done according to Hsu and colleagues [38]. Briefly, the intensity of staining was scored from 0–3, with 0 representing no staining, 1 indicating weak, 2 medium and 3 strong staining. The extent of staining was scored as 1 indicating < 25% of tumour area stained; 2 indicating 25–75% of area stained; and 3 indicating > 75% area stained. These two scores were summed to give each specimen a final score for use in the statistical analysis.

Statistical analysis

Variables used in the statistical analysis included p68 kinase staining score (0–6) before and during treatment, overall survival and response duration (in months) and growth inhibition in cell lines by [^{3}H]-thymidine incorporation

Table 1. *Antiproliferative effects of IFN-α* (n = 3)

Cell line	IFN-α (IU/ml)	Growth inhibition (%)⋆	*P* value†
Bon1	0	–	
	10	13.3	NS
	100	24.5	< 0.005
	1000	38.1	< 0.002
	10 000	48.0	< 0.001
LCC18	0	–	
	10	15.0	NS
	100	23.4	< 0.01
	1000	38.3	< 0.005
	10 000	45.5	< 0.001

⋆24 h incubation with the indicated concentration of IFN-α. †Statistical significance by Student's *t* test compared to untreated control.

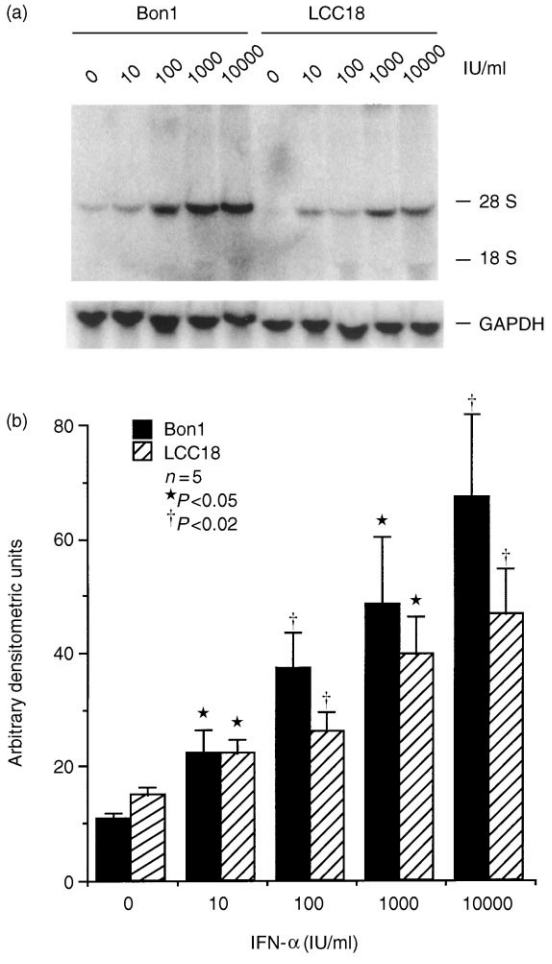

Figure 1. Effect of IFN-α concentration on p68 mRNA expression in Bon1 and LCC18 cell lines. (a) Northern blot analysis following hybridisation with the p68 cDNA, the membrane was rehybridised with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA control. Positions of the 28 and 18 S rRNAs are indicated. (b) Densitometric values expressed as arbitrary units with GAPDH values = 1. P68 expression in both cell lines were increased with graded concentrations of IFN-α. Statistical significance was analysed by Student's *t* test compared with the control.

ratio. Differences in p68 kinase score before and during treatment were calculated with the Student's *t* test. Response duration and overall survival probabilities were estimated based using Kaplan–Meier's Plot and Log rank test. *P* values < 0.05 were considered as significant.

RESULTS

Cell lines

Antiproliferative effect of IFN-α by [^{3}H]thymidine incorporation. IFN-α showed a significant dose-dependent antiproliferative effect on both Bon1 and LCC18 cell lines. The growth inhibition of both cell lines was in the range of 13–48%. Both cell lines were sensitive to IFN-α even at comparatively low concentration (100 IU/ml medium). Experiments were repeated three times (Table 1).

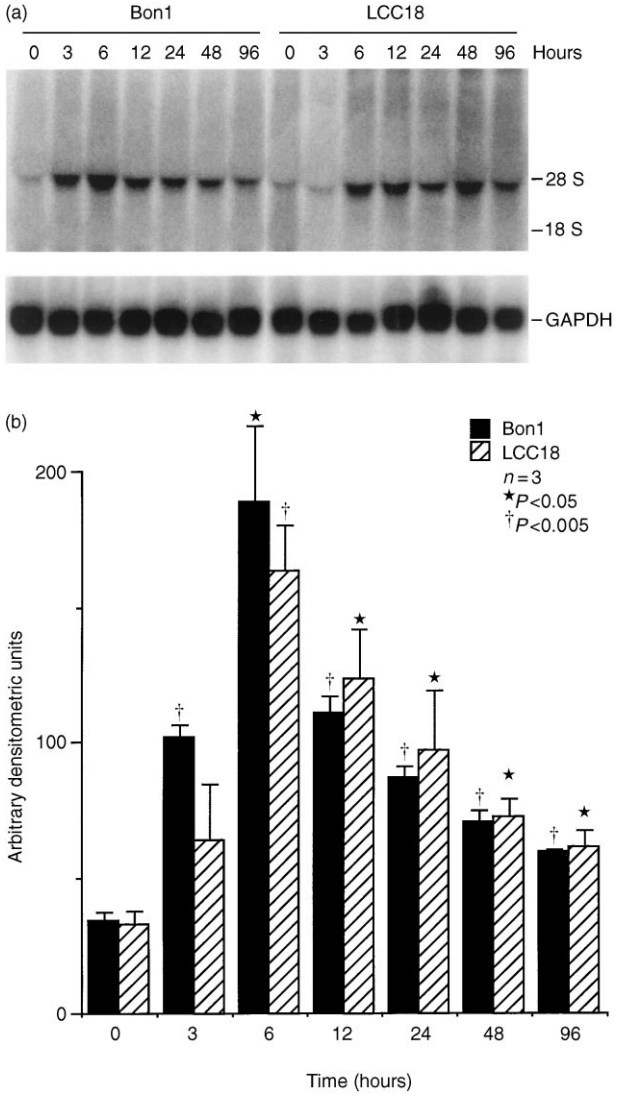

Figure 2. Effect of 1000 IU/ml IFN-α on p68 mRNA expression over time in Bon1 and LCC18 cell lines. (a) Northern blot analysis following hybridisation with the p68 cDNA, the membrane was rehybridised with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA control. Positions of the 28 and 18 S rRNAs are indicated. (b) Densitometric values expressed as arbitrary units with GAPDH values = 1. After exposure to IFN-α for 3 h, p68 mRNA expression is significantly increased in both cell lines. At 6 h exposure to IFN-α p68 mRNA expression reached a maximum in both cell lines. Statistical analysis was done by Student's *t* test compared with the control.

P68 kinase mRNA expression. The induction of p68 kinase mRNA by IFN-α was dose dependent (Figure 1a). At 12 hours of IFN-α exposure, the induction was approximately linear to the logarithm of IFN-α concentration. P68 mRNA was significantly induced even at low IFN-α concentrations (10 IU/ml; Figure 1b).

The kinetics regarding IFN-α induced expression of p68 kinase mRNA is shown in Figure 2(a). The maximal level of p68 mRNA expression (4- to 6-fold increase) was observed 6 h after the addition of IFN-α (1000 IU/ml). The levels then decreased, and remained at a lower level (2- to 3-fold increase) up to 96 h (Figure 2b). The same induction pattern of p68 kinase mRNA was observed at days 1 to 3, if a new dose of IFN-α was given repeatedly (data not shown). 2-5OAS and Mx-A protein mRNA were induced similarly (data not shown).

P68 kinase protein expression. To investigate IFN-α mediated enhancement of p68 kinase protein expression, 10 μg of the total protein extracts from Bon1 and LCC18 cells grown for 72 h in the absence and presence of IFN-α (100–10 000 IU/ml) were analysed by Western blot. The induction of p68 kinase protein by IFN-α was dose dependent (Figure 3). The time course of IFN-α mediated enhancement of p68 kinase protein expression were determined by Western blot. After 12 h of IFN-α treatment (2,000 IU/ml), a slight increase and after 24–48 h a strong increase in the amounts of p68 kinase protein could be observed. A longer exposure to IFN-α than 48 h did not result in a further increase (data not shown).

Tumour specimens

P68 kinase expression by immunohistochemistry. To analyse the effects of IFN-α therapy on p68 kinase expression, 99 samples representing 56 patients with carcinoid tumors were stained with anti-p68 using MAb TJ4C4. Immunohistochemical staining of p68 MAb is shown in Figure 4. After treatment with IFN-α p68 staining was significantly stronger than before treatment (Table 2). There was no significant correlation with the duration of IFN-α therapy (data not shown) and no differences were seen between primary and metastatic tumours (data not shown).

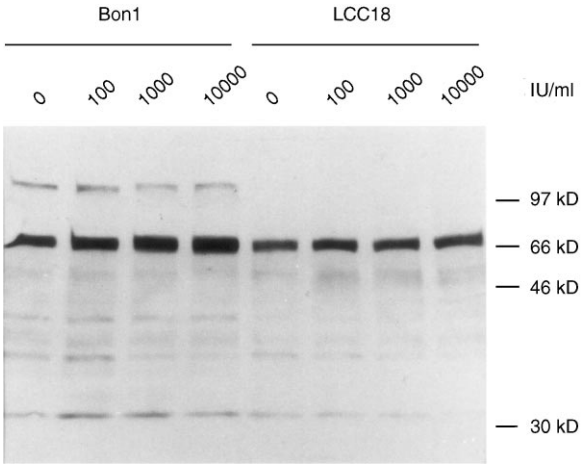

Figure 3. Western blot analysis of p68 kinase in Bon1 and LCC18 cell lines. The cells were treated with IFN-α at various concentrations of IFN-α (lane 1, 0; lane 2, 100; lane 3, 1,000; lane 4, 10 000 IU/ml). After 72 h of culture, cells were collected and Western blot analysis was done as described in Materials and Methods.

Correlation of p68 kinase score and treatment response. To determine the relationship between treatment response and p68 score, we divided the patients into three groups, clinical response, stable disease and tumour progression according to the clinical state when the tumour specimen was taken. In general, the p68 score increased in all three groups. However, there were statistical differences only between the before and after treatment groups with those patients having stable disease or tumour response (Table 2).

Evaluation of the prognostic significance of p68 kinase score. The medium survival of patients was 79.1 months (range, 12–199 months) from the start of IFN-α treatment. The median duration of disease stabilisation and/or response was 29.4 months (range, 3–145 months). The probabilities for

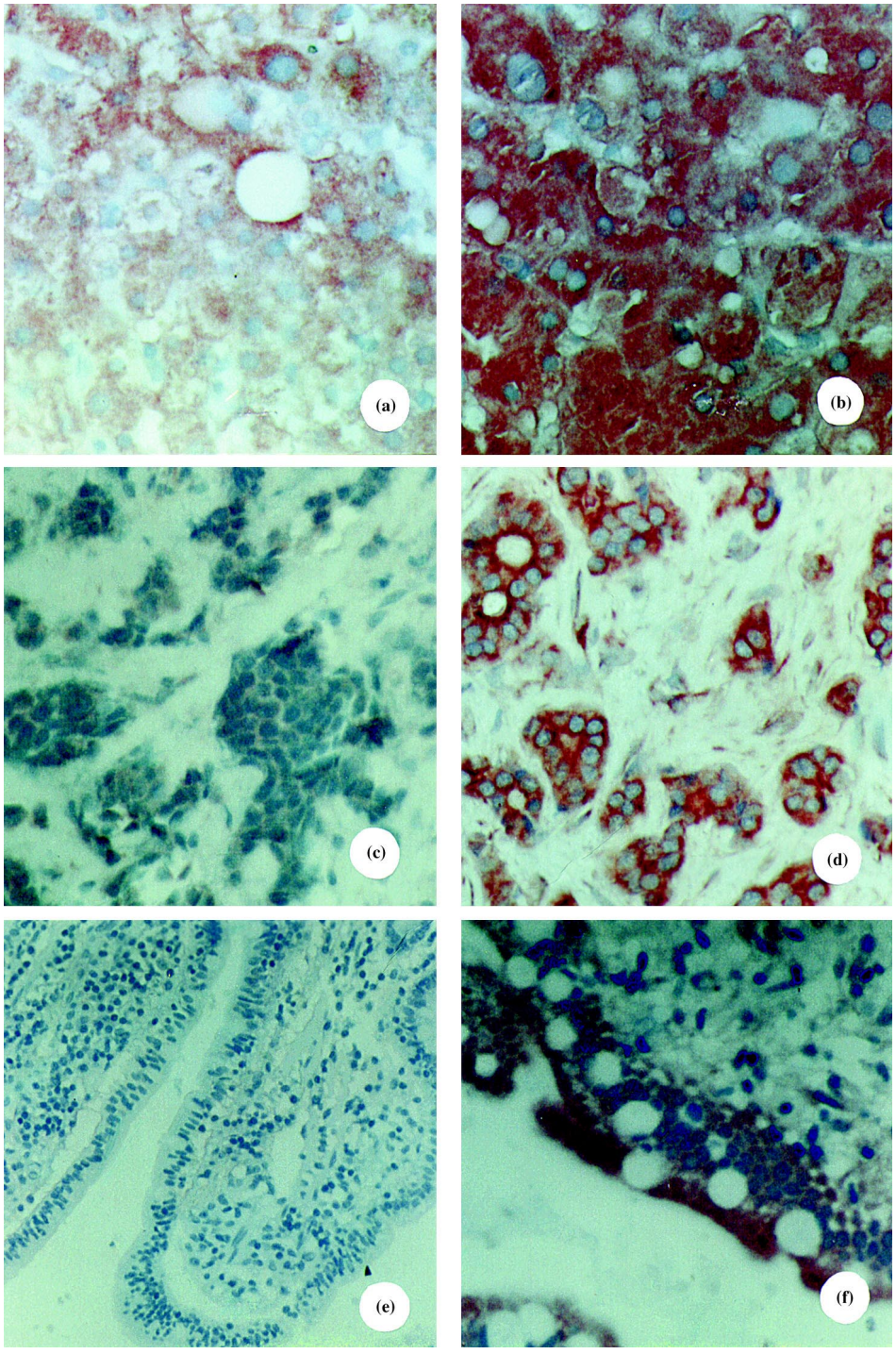

Figure 4. Immunohistochemical staining of carcinoid tumours with the anti-p68 kinase MAb TJ4C4. (a) Liver metastasis of a mid-gut carcinoid tumour showing moderate cytoplasmatic staining, before treatment. ×400 (b) Liver metastasis after 6 months treatment with IFN-α showing intensive staining (from the same patient (a)) ×400 (c) Weak p68 immunoreactivity of liver metastasis, before treatment. ×400 (d) Lymph node metastasis with strong cytoplasmatic staining, after treatment. ×400 (e) Small intestine without p68 staining of epithelial cells, before treatment. ×200 (f) Small intestine with moderate staining of epithelial cells, before treatment. ×400.

Table 2. Relationship between P68 score, IFN-α treatment time (month) and response

Group	No. of patients	Before treatment	During treatment	P*†
Treatment period				
< 6	7	2.61 ± 0.25	3.49 ± 0.32	< 0.01
> 6– ≤ 12	14	2.90 ± 0.19	3.56 ± 0.21	< 0.001
> 12– ≤ 24	21	2.77 ± 0.21	3.45 ± 0.20	< 0.001
> 24– ≤ 36	24	2.85 ± 0.19	3.46 ± 0.17	< 0.002
> 36– ≤ 48	29	2.84 ± 0.17	3.52 ± 0.16	< 0.001
Total	38	2.72 ± 0.15	3.47 ± 0.12	< 0.001
Clinical status				
Stable disease	29	2.71 ± 0.19	3.40 ± 0.14	< 0.001
Response	7	3.13 ± 0.22	4.00 ± 0.24‡	< 0.05
Progress	5	2.32 ± 0.24	2.86 ± 0.26‡	NS

*Paired *t* test. †Student's *t* test. ‡$P < 0.02$ (response versus progres-

overall survival ($P < 0.0001$) or time until progression ($P = 0.0062$) were significantly lower in the patients whose p68 kinase score was less than 3.0 (Figure 5).

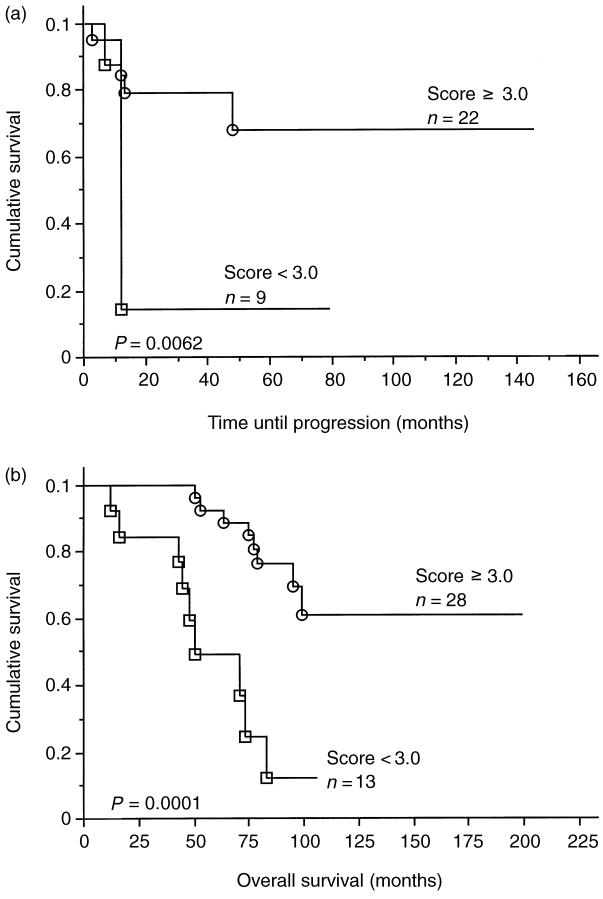

Figure 5. Probability of (a) progression-free survival and (b) overall survival according to p68 scores either < 3.0 or ≥ 3.0 (*n*=number of cases). Patients with p68 score ≥ 3.0 had a significantly longer progression-free survival than those with p68 score < 3.0 (log-rank test, $P = 0.0062$), and a longer overall survival ($P = 0.0001$).

DISCUSSION

Classical malignant carcinoid tumours are generally slow growing neoplasms. However, due to excessive production of biologically active substances, the quality of life is significantly impaired and untreated patients with the carcinoid syndrome and liver metastasis frequently die within 2–3 years from diagnosis [23, 39]. Chemotherapy has provided little benefit, altering response rates by only 0–30% [23]. Somatostatin analogues provide effective control of clinical symptoms, but have limited effect on tumour growth [23, 39, 40]. In several studies IFN-α has produced responses as measured by biochemical markers in approximately 50% of the patients, and significant tumour reduction and disease stabilisation in approximately 70–80% of patients [9, 23, 26, 37–41]. Since approximately one third of the patients might not benefit from this kind of treatment and this therapy has significant side-effects, it is of great importance to find indicators of possible response to IFN-α therapy. Induction of 2-5OAS as such a marker was shown by Grander and colleagues [6]. The analysis of 2-5OAS in human tissue is cumbersome, expensive and not suitable for routine clinical use. Therefore, we decided to look for other markers of IFN-α bioactivity which correlates with therapeutic outcome. The induced levels of p68 kinase (PKR) by IFN-α have, in some recent studies, in other tumour types correlated with antitumour response and overall survival [15, 16, 42].

Our study indicates that using the MAb (TJ4C4), we could correlate the antitumour response as well as overall survival to the extent of staining for p68. Using a threshold level of 3 for immunostaining, a significant difference for progression-free ($P = 0.0062$) and overall survival ($P < 0.0001$) was identified. Therefore, this immunohistological method, using formalin fixed paraffin embedded material, can be readily applied to routine clinical specimens and is of practical clinical value for predicting the therapeutic outcome to IFN-α. A future application of these findings may be to assess the biological responsiveness of fresh biopsies cultured short term to different concentrations of IFN-α. This approach may provide valuable information within 24 h and further refine the dosage of IFN-α needed to be given to a particular patient to achieve a biological response.

The assessment of mRNA expression of p68 kinase in two different cell lines which were sensitive to IFN-α treatment showed a maximal induction within 6–9 h and was dose related. This induction also correlated with inhibition of tumour growth in a dose-dependent manner. This was noted at low concentrations of IFN-α (10–100 IU/ml) which is clinically relevant and which resulted in a significant expression of p68 kinase mRNA. This finding is significant because it opens the possibility of designing treatment schedules based on the biologically relevant doses rather than on a predetermined clinical dose.

In conclusion, this study has demonstrated that IFN-α is able to induce p68 kinase at the mRNA and protein levels both *in vitro* and in a clinical setting. p68 protein expression correlates with clinical outcome of IFN-α therapy and was a predictor of the duration of response and overall survival. These findings are in agreement with previous work on human Kaposi's sarcoma and in head and neck squamous cell carcinomas [16, 17].

1. Uze G, Lutfalla G, Mogensen KE. Alpha and beta interferons and their receptor and their friends and relations. *J Interferon Cytokine Res* 1995, **15**, 3–26.
2. Hovanessian AG. Interferon-induced and double-stranded RNA-activated enzymes: a specific protein kinase and

2′,5′-oligoadenylate synthetases. *J Interferon Res* 1991, **11**, 199–205.
3. Samuel CE. Antiviral actions of interferon. Interferon-regulated cellular proteins and their surprisingly selective antiviral activities. *Virology* 1991, **183**, 1–11.
4. Lengyel P. Tumor-suppressor genes: news about the interferon connection. *Proc Natl Acad Sci USA* 1993, **90**, 5893–5895.
5. Hovanessian AG. The double stranded RNA-activated protein kinase induced by interferon: dsRNA-PK. *J Interferon Res* 1989, **9**, 641–647.
6. Grander D, Oberg K., Lundqvist ML, *et al.* Interferon-induced enhancement of 2′,5′-oligoadenylate synthetase in mid-gut carcinoid tumours [see comments]. *Lancet* 1990, **336**, 337–340.
7. Sen GC, Lengyel P. The interferon system. A bird's eye view of its biochemistry. *J Biol Chem* 1992, **267**, 5017–5020.
8. Taylor JL, Samuel CE, Grossberg SE. Antiviral and antidifferentiative activities of interferon beta and gamma in relation to their induction of double-stranded RNA-dependent protein kinase activity in 3T3-L1 cells. *J Biol Chem* 1988, **263**, 1336–1341.
9. Oberg K. The action of interferon alpha on human carcinoid tumours. [Review]. *Semin Cancer Biol* 1988, **3**, 35–41.
10. Lee SB, Melkova Z, Yan W, Williams BR, Hovanessian AG, Esteban M. The interferon-induced double-stranded RNA-activated human p68 protein kinase potently inhibits protein synthesis in cultured cells. *Virology* 1993, **192**, 380–385.
11. Williams BR. Signal transduction and transcriptional regulation of interferon-alpha-stimulated genes. *J Interferon Cytokine Res* 1991, **11**, 207–213.
12. Koromilas AE, Roy S, Barber GN, Katze MG, Sonenberg N. Malignant transformation by a mutant of the IFN-inducible dsRNA-dependent protein kinase. *Science* 1992, **257**, 1685–1689.
13. Lee SB, Esteban M. The interferon-induced double-stranded RNA-activated protein kinase induces apoptosis. *Virology* 1994, **199**, 491–496.
14. Barber GN, Wambach M, Wong ML, Dever TE, Hinnebusch AG, Katze MG. Translational regulation by the interferon-induced double-stranded-RNA-activated 68-kDa protein kinase. *Proc Natl Acad Sci USA* 1993, **90**, 4621–4625.
15. Haines GK, Ghadge G, Thimmappaya B, Radosevich JA. Expression of the protein kinase p-68 recognized by the monoclonal antibody TJ4C4 in human lung neoplasms. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1992, **62**, 151–158.
16. Haines GK, Becker S, Ghadge G, Kies M, Pelzer H, Radosevich JA. Expression of the double-stranded RNA-dependent protein kinase (p68) in squamous cell carcinoma of the head and neck region. *Arch Otolaryngol Head Neck Surg* 1993, **119**, 1142–1147.
17. Haines GK, Ghadge GD, Becker S, *et al.* Correlation of the expression of double-stranded RNA-dependent protein kinase (p68) with differentiation in head and neck squamous cell carcinoma. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1993, **63**, 289–295.
18. Haines GK, Ghadge G, Radosevich JA. Expression of p68 protein kinase as recognized by the monoclonal antibody TJ4C4 during human fetal development. *Tumour Biol* 1993, **14**, 95–104.
19. Singh C, Haines GK, Talamonti MS, Radosevich JA. Expression of p68 in human colon cancer. *Tumour Biol* 1995, **16**, 281–289.
20. Wood JN, Hovanessian AG. Interferon enhances 2-5A synthetase in embryonal carcinoma cells. *Nature* 1979, **282**, 74–76.
21. Meurs EF, Galabru J, Barber GN, Katze MG, Hovanessian AG. Tumor suppressor function of the interferon-induced double-stranded RNA-activated protein kinase. *Proc Natl Acad Sci USA* 1993, **90**, 232–236.
22. Bajetta E, Di BM, Zilembo N, Bochicchio AM. Medical treatment of neuroendocrine tumors. *Tumori* 1993, **79**, 380–388.
23. Oberg K. Chemotherapy and biotherapy in neuroendocrine tumors [Review]. *Current Opinion in Oncology* 1993, **5**, 110–120.
24. Elvin A, Oberg K, Lindgren PG, *et al.* Interferon treatment of neuroendocrine tumour xenografts as monitored by MRI. *Acta Oncologica* 1994, **33**, 567–571.
25. Buer J, Kirchner H, Schomburg A, *et al.* Cytokine-based biotherapy of gastrointestinal tumors. *Clin Invest* 1994, **72**, 526–534.
26. Jacobsen MB, Hanssen LE, Kolmannskog F, Schrumpf E, Vatn MH, Bergan A. Interferon-alpha 2b, with or without prior hepatic artery embolization: clinical response and survival in mid-gut carcinoid patients. The Norwegian carcinoid study. *Scand J Gastroenterol* 1995, **30**, 789–796.
27. Chaudhry A, Oberg K, Wilander E. A study of biological behavior based on the expression of a proliferating antigen in neuroendocrine tumors of the digestive system. *Tumour Biology* 1992, **13**, 27–35.
28. Waltenberger J, Lundin L, Oberg K, *et al.* Involvement of transforming growth factor-beta in the formation of fibrotic lesions in carcinoid heart disease. *Am J Path* 1993, **142**, 71–78.
29. Stridsberg M, Hellman U, Wilander E, Lundqvist G, Hellsing K, Oberg K. Fragments of chromogranin A are present in the urine of patients with carcinoid tumours: development of a specific radioimmunoassay for chromogranin A and its fragments. *J Endocrinol* 1993, **139**, 329–237.
30. Stridsberg M, Lundqvist G, Engstrom U, *et al.* Development of polyclonal antibodies and evaluation of a sensitive radioimmunoassay for detection and measurement of synaptophysin. *Acta Neuropathologica* 1994, **87**, 635–641.
31. Stridsberg M, Oberg K, Li Q, Engstrom U, Lundqvist G. Measurements of chromogranin A, chromogranin B (secretogranin I), chromogranin C (secretogranin II) and pancreastatin in plasma and urine from patients with carcinoid tumours and endocrine pancreatic tumours. *J Endocrinol* 1995, **144**, 49–59.
32. Stridsberg M, Eriksson B, Lundqvist G, Skogseid B, Wilander E, Oberg K. Islet amyloid polypeptide (IAPP) in patients with neuroendocrine tumours. *Regulatory Peptides* 1995, **55**, 119–131.
33. Evers BM, Ishizuka J, Townsend CJ, Thompson JC. The human carcinoid cell line, BON. A model system for the study of carcinoid tumors. *Ann NY Acad Sci* 1994, **733**, 393–406.
34. Lundqvist M, Mark J, Funa K, *et al.* Characterisation of a cell line (LCC-18) from a cultured human neuroendocrine-differentiated colonic carcinoma. *Eur J Cancer* 1991, **27**, 1663–1668.
35. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987, **162**, 156–159.
36. Meurs E, Chong K, Galabru J, *et al.* Molecular cloning and characterization of the human double-stranded RNA- activated protein kinase induced by interferon. *Cell* 1990, **62**, 379–390.
37. Tiensuu JE, Ahlstrom H, Andersson T, Oberg K. Octretide and interferon alpha: a new combination for the treatment of malignant carcinoid tumours. *Eur J Cancer* 1992, **28A**, 1647–1650.
38. Hsu DW, Efird JT, Hedley WE. Prognostic role of urokinase-type plasminogen activator in human gliomas. *Am J Pathol* 1995, **147**, 114–123.
39. Oberg K. Endocrine tumors of the gastrointestinal tract: systemic treatment [Review]. *Anti Cancer Drugs* 1994, **5**, 503–519.
40. Oberg K, Eriksson B, Janson ET. Interferons alone or in combination with chemotherapy or other biologicals in the treatment of neuroendocrine gut and pancreatic tumors [Review]. *Digestion* 1994, **3**, 64–69.
41. Janson ET, Ronnblom L, Ahlstrom H, *et al.* Treatment with alpha-interferon versus alpha-interferon in combination with streptozocin and doxorubicin in patients with malignant carcinoid tumors: a randomized trial. *Ann Oncol* 1992, **3**, 635–638.
42. Buffet JC, Vannier JP, Laurent AG, Robert N, Hovanessian AG. Enhanced level of double-stranded RNA-dependent protein kinase in peripheral blood mononuclear cells of patients with viral infections. *J Interferon Res* 1986, **6**, 85–96.

Acknowledgements—This study was supported by Sweden Cancer Research Fund and Lions Cancer Research Fund to (K.O.) and a Veterans Affairs Merit Review Grant to (J.A.R.).