Acta Oncologica Vol. 35, No. 4, pp. 473–478, 1996

EFFECTS OF INTERFERONS AND TUMOUR NECROSIS FACTOR-α ON HUMAN LUNG CANCER CELL LINES AND THE DEVELOPMENT OF AN INTERFERON-RESISTANT LUNG CANCER CELL LINE

EDUARDO SUAREZ PESTANA, GUNILLA BJÖRKLUND, ROLF LARSSON, PETER NYGREN, KENNETH NILSSON and JONAS BERGH

Thirteen human lung cancer cell lines, 7 representing small cell lung cancer (SCLC) and 6 different types of non-SCLC, were tested for sensitivity to tumour necrosis factor alpha (TNF-α) and interferon alpha and gamma (IFN-α and γ) using an automated fluorometric microculture cytotoxicity assay (FMCA). One SCLC line (H-82) was found to be sensitive to IFN-α in short-term (72 h) culture, whereas after prolonged (5 days) culture two additional SCLC cell lines responded to IFN-γ. TNF-α inhibited the growth of one large cell carcinoma cell line (H-157), whereas all SCLC lines were found to be insensitive. The combination of IFN-γ and TNF-α produced no further response compared with the single agents used alone. By continuous cultivation of the IFN-α-sensitive cell line H-82 in the presence of increasing concentrations of IFN-α, an IFN-α-resistant subline (H-82R) was established. This line displayed a high degree of resistance (>100 fold) to IFN-α and cross-resistance to IFN-γ. There was no alteration in the number of IFN binding sites, in the growth rate, the expression of selected surface markers for SCLC or the expression of multidrug resistance markers in the H-82R subline compared with the parental H-82 cell line. The results demonstrate a heterogeneous response of SCLC cell lines to IFN-α and γ and TNF-α with only a minority of the cell lines responding to these agents by growth inhibition. The IFN-α and γ H-82R subline may serve as a valuable tool in future studies on the mechanisms of IFN antitumour activity.

Human lung cancer can be categorized as two major groups, small (SCLC) and non-small cell lung cancer (non-SCLC) (1). This distinction is based on morphological characteristics, biochemical marker profile and response to radio- and chemotherapy (1). Seventy percent of the lung cancers fall into the non-SCLC group which can be subdivided into three histopathological entities; squamous cell carcinoma (SQC), adenocarcinoma (ADC) and large cell carcinoma (LCC) (1). These subgroups are defined by different epithelial markers and a poor to moderate response to external beam radiotherapy and chemotherapy. The SCLC group is characterized by the expression of several neuroendocrine markers and by an initial prompt response to oncological therapy modalities (1). Chemotherapy significantly prolongs the survival of SCLC (1). However, only 1–5% of the SCLC patients become long-term survivors (1). Better survival figures have been presented from some institutions, especially in patients with 'limited' SCLC at diagnosis. Some 10% of the non-SLLC tumours also expresses neuro-endocrine markers at higher levels compared with other tumours in the non-SCLC group (2).

Because of the poor clinical results in the management of lung cancer patients there is a need for new therapeutic

Received 9 December 1994.
Accepted 10 July 1995.
From the National Institute for Oncology and Radiobiology, Havana, Cuba (E. Suarez Pestana), Departments of Pathology (G. Björklund, K. Nilsson), Clinical Pharmacology (R. Larsson, P. Nygren) and Oncology (P. Nygren, J. Bergh), University Hospital, Uppsala, Sweden.
Correspondence to: Dr Jonas Bergh, Dept of Oncology, Akademska sjukhuset, Uppsala University, S-751 85 Uppsala, Sweden

 ISSN 0284-186X

Table 1

Growth characteristics and references for the human lung cancer cell lines used in this study

Cell line	Class[1]	Serum	Growth[2]	Reference
NCIH-69	SCLC	NCS	Susp/att	8*
NCIH-82	SCLC	NCS	Susp/att	8*
U-1285	SCLC	NCS	Susp	9
U-1568	SCLC	FCS	Susp	9
U-1690	SCLC	NCS	Susp/att	10
U-1906	SCLC	NCS	Susp/att	10
U-2020	SCLC	NCS	Susp/att	10
NCIH-23	ADC	NCS	Adh	8*
NCIH-125	ADC	NCS	Adh	8*
NCIH-157	LCC	NCS	Adh	8*
NCIH-661	LCC	NCS	Adh	*
U-1810	LCC	NCS	Adh	10
U-1752	SQC	NCS	Adh	11

[1] Classification: SCLC = small cell carcinoma, ADC = adenocarcinoma, LCC = large carcinoma and SQC = squamous cell carcinoma, FCS = foetal calf-serum, NCS = newborn calf-serum.
[2] Susp = suspension, Att = attached, Adh = adherent (monolayer).
* Generous gift from Drs Minna and Gazdar.

strategies, which may require the use of cytokines. Tumour necrosis factor-alpha (TNF-α) has been shown to have an antitumour effect in murine tumours and human tumour xenografts in vivo (3) and to be cytotoxic to many types of human tumour cell lines in vitro (4). The response of the cell lines was heterogeneous with sensitivity in only about one-third of the cell lines of a given tumour entity (5). Interferon-alpha IFN-α has been shown to inhibit the tumour growth of a fraction of different human cell lines in vitro and has shown clinical antitumour activity in a few human cancers, including hairy cell leukaemia, chronic myelogenous leukaemia, multiple myeloma and malignant lymphoma.

Relatively little is known about the effects of TNF-α and IFNs on lung cancer. The results of previous studies have suggested that human lung cancer cell lines in vitro only rarely are responsive to IFN-α and γ (6) and not to TNF-α (7).

In the present study we have investigated the effects of TNF-α and IFN-γ on a panel of 13 well-characterized cell lines representing different types of human lung cancer (8-11). The results indicate that three cell lines were sensitive to the IFNs and one responded to TNF-α. Furthermore, from one of the IFN-α-sensitive lines a resistant subline was established and characterized.

Material and Methods

Cell cultures

For this study we used 13 established lung cancer cell lines, previously diagnosed as 7 SCLC, and 6 non-SCLC cell lines respectively (Table 1). The NCI cell lines were generous gifts from Drs Minna and Gazdar (8) (present affiliation Simmon's Cancer Center, Dallas, Texas, USA). The stock cultures were grown in RPMI 1640 (Gibco Ltd., Grand Island, NY, USA) supplemented with 10% heat inactivated foetal or newborn calf serum (Gibco), 2 mM glutamine, 50 IU/ml penicillin and 25 μg/ml streptomycin and incubated in 37°C in humidified 5% CO_2_ in-air atmosphere. Fresh medium was added twice a week. All cell lines used were tested negative for mycoplasma by specific DNA probes by Sveriges Veterinärmedicinska Anstalt (Uppsala, Sweden). The cell viability was measured by the trypan blue exclusion method.

Establishment of an IFN-α-resistant SCLC cell line

To select an IFN-α-resistant cell line of the H-82, an IFN-α-sensitive small cell lung cancer cell line established by Carney et al. 1985 (8), we seeded the H-82 cells at a concentration of 0.2×10^6 cells/ml in RPMI 1640 (Gibco Ltd., Grand Island, NY, USA) supplemented with 10% foetal calf serum (FCS, Gibco), 2 mM L-glutamine, 50 IU/ml penicillin and 25 μg/ml streptomycin. Initially we added 1 IU/ml IFN-α2b IntronA (a generous gift from the Schering Corp., Bloomfield, NJ, USA). The concentration was then increased at intervals until we reached the final concentration of 1000 IU/ml two months later. The new IFNα-resistant cell line was designated H-82R. The IFN-α was subsequently removed from the medium in order to investigate the reversibility of the resistance. The new resulting subline was designated H-82R$^-$. The growth of the H-82, H-82R and H-82R-cell lines was assessed by seeding a concentration of 0.1×10^6 cells/ml in RPMI 1640 with the supplements described above plus 1 000 IU/ml of IFN-α for H-82R. The cell concentration was determined by counting in a Bürker chamber, and the viability by the trypan blue exclusion method.

Surface markers analysis

One million cells were washed three times in PBS + 2%FCS + 0.1%NaN_3, incubated for 30 min with the SCLC-specific antibodies; MOC-1 (12), TSF-2 (13) or F-12 (14), washed three times, incubated for 30 min with a FITC-conjugated anti-mouse antibody (Janssen Biochimica, Olen, Belgium), washed twice and finally measured in a FACScan (Becton-Dickinson, Moutain View, CA, USA). The whole procedure was performed on ice. The lung cancer cell line U-2050 was used as a positive control.

Measurement of cytokine sensitivity

The cells were seeded at a concentration of 10^4 cells per well in 96 wells plates (Costar, Cambridge, MA, USA) with or without cytokines at different concentrations. In all cases 9 replicates were made and plates were prepared for

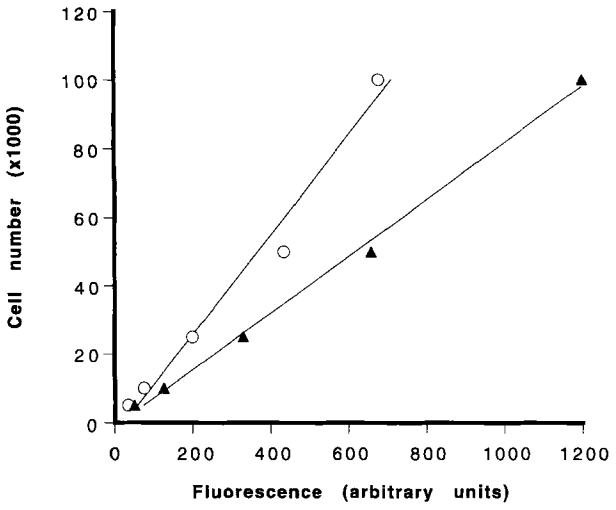

Fig. 1. Relationship between living cell number and FDA fluorescence for H-82R and U-1285. The indicated number of cells was seeded in a 96-well microtitre plate and subsequently analysed by Fluoroscan 2. The results are expressed as absolute fluorescence and presented as means of triplicate wells. (One typical experiment of three).

Table 2

IC 50s calculated from the fluorometric analysis. The time in culture (days) required to obtain the IC 50 is shown within parentheses

Cell line	IFN-α	IFN-γ	TNF-α	IFN-γ + TNF-α
H-69	>5000	>1000	>10	–
H-82	4.5 (3D)	>1000	>10	–
U-1285	>5000	>1000	>10	–
U-1568	ND	102 (5D)	>10	ND
U-1690	>5000	>1000	>10	–
U-1906	>5000	>1000 (5D)	>10	–
U-2020	>5000	95 (5D)	>10	–
H-23	>5000	>1000	>10	–
H-125	>5000	>1000	>10	–
H-157	>5000	>1000	6.9 (10D)	–
H-661	>5000	>1000	>10	–
U-1753	>5000	>1000	>10	–
U-1810	>5000	>1000	>10	–

Abbreviations: IC 50 = Concentration causing a 50% reduction in number of viable cells IFN-α-interferon α2B. IFN-γ = Interferon γ. TFN-α = Tumor necrosis factor α. ND = not done. D = Days. – = Effect less then produced by either cytokine done.

analysis at three different timepoints. All experiments were repeated three times. The concentrations of recombinant human IFN-α2b (IntronA; kindly donated by the Schering Corp., Bloomfield, NJ, USA) were 10, 100, 1 000 and 5 000 IU/ml; of recombinant human IFN-γ (a generous gift from the Interferon and Biotechnology Center, Havana, Cuba) 10, 100 and 1 000 IU/ml; and of recombinant human TNF-α (generously donated by Dr GR Adolf of the Ernst-Boehringer Inst., Vienna, Austria) 0.1, 1 and 10 ng/ml. The concentrations in the combination study with IFN-γ and TNF-α were 10 IU/ml and 0.1 ng/ml respectively. The fluorometric analysis was performed on days 0, 3, 7 and 10 in the experiments with IFN-α2b and TNF-α, and on days 0, 3, 5 and 7 with IFN-γ and IFN-γ/TNF-α. The histocytic lymphoma cell line U-937 (15) was used as a TNF-α and IFN-γ sensitive control.

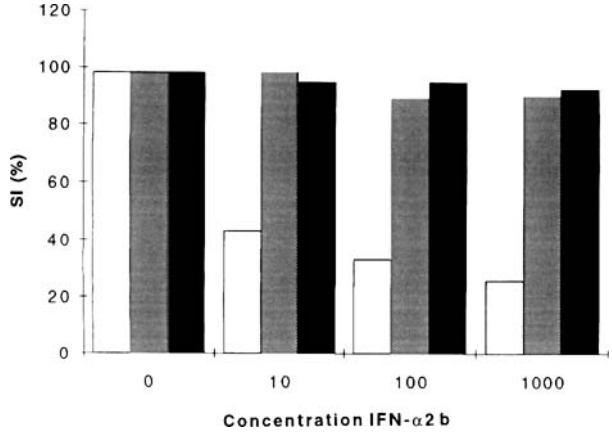

Fig. 2. Effect of increasing concentrations of IFN-α on SI in H-82 (□), H-82R (□) and H-82REV. (■). (One typical experiment of three).

Cell survival after culture in the presence and absence of cytokines was determined by using the fluorometric microculture cytotoxicity assay (FMCA) as described elsewhere (16). The assay is based on hydrolysis of non-fluorescent fluorescein diacetate (FDA; Sigma Chem. Co., St Louis, MO, USA) to a strongly fluorescent product by viable cells with intact plasma membranes (16). Briefly, after 72 h of microtitre plate culture, the plates were centrifuged (200 g, 5 min), the medium removed by flicking the plate, and the wells washed once with 200 μl PBS. PBS (200 μl) with 10 μg/ml FDA was then added to each well. Each experimental condition as well as control culture without cytokines consisted of nine replicate wells. The plates were incubated for 60 min at 37°C after which the fluorescence from each well was read in a Fluoroscan II microfluorometer (Labsystems OY, Helsinki, Finland) with filters set at 485 and 538 nm for excitation and emission, respectively. FMCA results were found to correlate well with those obtained with the established MTT and differential staining cytotoxicity (DiSC) assay (17,18). The results are presented as Survival Index (SI): FDA(t)/FDA(c) × 100 with blank values subtracted, where t and c denote test and control wells, respectively. IC 50 was defined as the drug concentration giving an SI of 50% of control. All experiments were repeated at least three times.

Interferon-α receptor analysis

Equilibrium binding assay was performed by serially diluting the radiolabelled IFN-α (generously donated by E.

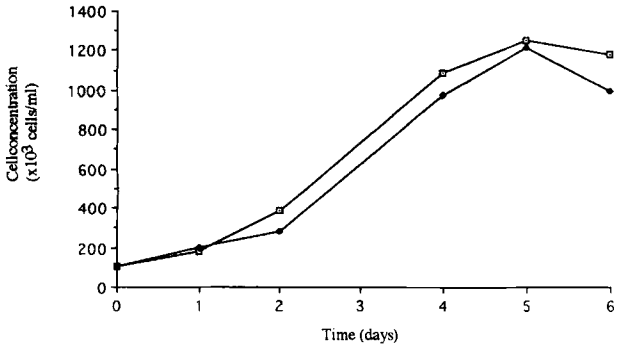

Fig. 3. Growth of H-82 and H-82R cells. Growth of the H-82 cell lines was assessed by seeding of 0.1×10^6 cells/ml in RPMI 1640 with the supplements described in material and methods plus 1 000 IU/ml of IFN-α for H-82R. The cell concentration was determined by counting in a Bürker chamber at indicated time-points. (One experiment out of three). -⊡- H82; -◆- H82 R.

Lundgren, Umeå) 1:2 in eight steps. IFN-α-containing medium (100 μl) was layered on the top of a two-step gradient in a 400 μl tube (Labconinc, San Rafael, CA, USA). The gradient consisted of 100 μl of 15% silicon oil (No. 17.563-3, Aldrich Chemical Co., Milwaukee, WI, USA) and 85% mineral oil (Sigma) and 100 μl of 10% sucrose in FCS. One million cells in an 0.1 ml culture medium were added and the tubes were incubated with intermittent rotation at room temperature. After 90 min the tubes were centrifuged and the tips of the tubes containing the cell pellets were cut off. The supernatant fractions were collected and counted separately from the cell pellets in a gamma counter (Beckman Gamma 8000). The calculated values of the number of binding sites per cell and the ligand affinity were determined by Scatchard analysis of equilibrium binding data, after subtraction of unspecific binding, determined in the presence of a 100-fold molar excess of unlabelled IFN-α. The assay was performed at least three times. The IFN-α receptor expressing Burkitt's lymphoma cell line Daudi (19) was used as positive control.

Results

Screening of IFN and TNF activity

The growth characteristics of the lung cancer cell lines used are summarized in Table 1. There was a linear relationship between cell number and FDA fluorescence, as illustrated for U-1285 and H-82R in Fig. 1. The SI values calculated from the fluorometric assay corresponded well with viability measurements carried out by trypan blue exclusion assay for H-82 and H-82R (data not shown). Of six SCLC lines tested, ony the H-82 responded to IFN-α with cytotoxicity in a dose-dependent manner with an IC 50 of <10 U/ml after 3 days in culture (Fig. 2, Table 2). By day 10, 90% of the cells incubated with 10 U/ml had died (not shown). Among the 6 non-SCLC lines tested, no significant responses were found, irrespective of length of time in culture. Two out of seven SCLC cell lines showed a more than 50% decrease in SI in response to IFN-γ (Table 2). The SI value at 1 000 U/ml was 57% on day 3 (Fig. 3). One large cell carcinoma cell line, the H-157, was sensitive to TNF-α. This cell line required a dose of more than 3.4 ng/ml TNF-α for a response in excess of 50% by day 10 (Table 2). No response was obtained for the SCLC lines. When a combination of INF-α and TNF-α was tested no further effect was observed compared with that obtained with either of the cytokines alone (Table 2).

Table 3

Immunofluorescence data

Antibody	H-82	H-82R	U-2050
MOC-1	+++	++	++
TSF-2	–	–	+
F-12	–	–	(+)

+++ >75% of the cells are positive. ++ 50–75% positive, +25–50% positive, (+) 10-25% positive. – negative for the lung cancer markers

Comparative studies on the characteristics of the IFN-α-subline H-82R and its parental cell line H-82

General phenotypic properties of the IFN-α-resistant subline H-82R. No difference in morphology was found between the parental H-82 and the subline H-82R. Both cell lines had phenotypic characteristics corresponding to those of a poorly differentiated SCLC. The growth rate and terminal cell density are also the same for the two cell lines (Fig. 3). Both cell lines were found to have a population doubling time of about 28 h and reached the plateau of the cell growth curve at 1.2×10^6 cells/ml. H-82 and H-82R were strongly positive for the anti-SCLC monoclonal antibodies MOC-1 (78 and 74% respectively) (10) and negative for TFS-2 and F-12 (Table 3) (12–14). No expression of the multidrug resistance-associated 170 kD protein Pgp

Table 4

Number of binding sites and affinities for IFNα-receptors on H-82 wildtype and sublines

Cell line	Sites/cell	K_d (pM)
H-82	1796	120
H-82R	2930	408
H-82R⁻	2420	231
Daudi	8107	251

170 was observed, as judged by immunohistochemical analysis using the Pgp 170 reactive C219 (20) (Centocor) antibody (not shown). Dose-response analysis of vincristine, etoposide and doxorubicin sensitivity using the fluorometric assay did not reveal any difference between the two cell lines (not shown).

IFN-α and γ and TNF-α sensitivity The parental cell line, H-82, is very sensitive to IFN-α (Fig. 2). After exposure of the H-82 to 10 IU/ml IFN-α for three days in culture a concentration of 10 IU/ml, the viability was increased by about 60%. The H-82R had developed a resistance to 1000 IU/ml IFN-α after two months (Fig. 2). Throughout the experiments, H-82R had a viability of 95%, which is the same as that for the control cells without IFN-α.

The H-82R was subsequently cultured without IFN-α (H-82R$^-$) and tested one month after the removal of IFN for IFN-α and γ sensitivity with the fluorometric assay. No change in the IFN resistance was observed (Fig. 2 and data not shown). H-82R was found to be resistant not only to IFN-α, but also to IFN-γ (data not shown). The resistance to TNF-α typical of H-82 in the parental cell line remained unchanged in the H-82R-subline.

IFN-α receptor analysis

The number of IFN-α binding sites did not change during the development of resistance. The H-82, H-82R and H-82R$^-$ cell lines all had expressed about 2 000 receptor sites/cell, which is one-quarter what is found in Daudi, with a K_d on $1-4 \times 10^{-10}$ M (Table 4).

Discussion

Our results on IFN-α, IFN-γ and TNF-α confirm previous data published by two independent groups on the respective effect of IFN and TNF on human lung cancer cells (6, 7). However, we cannot on the basis of this representative panel of human lung cancer cell lines confirm the synergistic, cytotoxic effect of IFN-γ and TNF-α described by Dubois et al. in 1989 (21). The most important observation in our study is the marked sensitivity of H-82 to IFN-α. The other seven SCLC cell lines were much less sensitive, including those lines with a similar marker profile to that of H-82. It has been proposed that H-82 belongs to a 'variant' group of SCLC (8). These 'variant' cell lines are characterized by lower levels of some neuroendocrine markers and have a less marked tendency to grow in dense clusters. The SCLC U-1285 and U-1906 partly fulfil these criteria, but were much less responsive to IFN-α. This provides further evidence of the previously demonstrated marked biological heterogeneity even within the morphologically distinct entity SCLC. In accordance with this, each tumour is unique in marker profile and most likely also in response to different therapeutical modalities. This is in accordance with the clinical reports demonstrating that a few SCLC patients have interferon-responding tumours (22).

The growth inhibitory/cytotoxic activity of drugs on a large number of human lung cancer cell lines measured by short-term in vitro assays has been found to reflect clinical experience (23). It has therefore been suggested that in vitro testing may be a valuable tool in the preclinical evaluation of new anticancer agents (23, 24). In the present study we have employed a fluorometric assay based on detection of esterase activity in cells with intact plasma membranes (16). Indeed, this assay system has been found to give promising individual clinical correlations (18).

The very marked effect of IFN-α on H-82 indicates that individual patients with SCLC may benefit from IFN-α2b treatment. However, the number of studies and patients included is too limited to permit any definite conclusions (25). On the other hand, our data can also be interpreted as a support for individualized therapy of SCLC after in vitro screening of new potential therapeutics. The feasibility of the present assay system for the in vitro identification of tumour-type specific drug activity using biopsy cells from patients has recently been demonstrated (24).

From the highly IFN-sensitive cell line H-82 we selected a stable, IFN-α-resistant cell line, which retains the same phenotypic characteristics as the parental line. There was no evidence of a multidrug resistant phenotype since neither cross-resistance to chemotherapeutic drugs nor expression of 170 KD P-glycoprotein was observed. The mechanism for IFN resistance in H-82R is unclear. Ucer et al. (26) suggest that the development of IFN-α resistance is due to a down-regulation of the IFN-α receptors. However, we found the same number of binding sites (2 000 sites/cell) with the same affinity (2×10^{-10} M) in both the sensitive and the resistant cell lines.

It has further been reported that IFN-α can even bind to IFN-α-resistant cells (27), but the complexes between IFN-α and its receptor are not coupled to the cytoskeletal matrix, which is the matter in a sensitive cell, and for that reason the signal is never internalized (28). We also found that the development of IFN-α resistance produced cross-resistance to IFN-γ. Since it is also well known that IFN-α and IFN-γ do not share the same receptor (29), the mechanism for development of resistance may be something other than the quantity of receptors. An alternative explanation for the co-resistance could be that IFN-α and IFN-γ share common second messengers, as exemplified by the 2′-5′oligo(A)synthetase, an enzyme mediating the antiviral and the antiproliferative effects of both types of interferon (30–32). H-82R was also cultured without IFN (H-82R$^-$). After one month in IFN-free medium, it still maintained the resistance to IFN. This cell line, in combination with H-82 and H-82R may be a useful tool for studies aimed at furthering our understanding of the mechanism of acquired resistance to IFN.

ACKNOWLEDGEMENTS

This project was supported by grants from the Swedish Cancer Society, Lions Fund at Akademiska sjukhuset and a fellowship from SAREC awarded to Suarez Pastena. We are grateful to Marléne Forslund for secreterial assistance.

REFERENCES

1. Ginsberg RT, Kris MG, Armstrong JG, Idhe DC, Pass H, Glatstein EJ. Cancer of the lung. de Vita VT Jr, Hellam S, Rosenberg S, eds. J.B. Lippencott Company, 1993: 673–758.
2. Linnoila RI, Mulshine J, Steinberg S, Funa K, Matthews MJ, Cotelingam J, Gazdar AF. Neuroendocrine differentiation in endocrine and nonendocrine lung carcinomas. Am J Clin Path 1988; 90: 1–12.
3. Asher A, Mule JJ, Reichert CM, Shiloni E, Rosenberg SA. Studies on the antitumor efficacy of systemically administered recombinant human tumor necrosis factor against several murine tumors in vivo. J Immunol 1987; 138: 963–74.
4. Wadler S, Schwartz EL. Antineoplastic activity of the combination of interferon and cytotoxic agents against experimental and human malignancies: A review. Cancer Res 1990; 50: 3473–86.
5. Frei III E, Spriggs D. Tumor necrosis factor: still a promising agent. J Clin Oncol 1989; 7: 291–4.
6. Twentyman PR, Workman P, Wright KA, Bleehen NM. The effects of α and γ interferons on human lung cancer cells grown in vitro or as xenografts in nude mice. Br J Cancer 1985; 52: 21–9.
7. Giaccone G, Kadoyama C, Maneckjee R, Venzon D, Alexander RB, Gazdar AF. Effects of tumor necrosis factor, alone or in combination with topoisomerase-II-targeted drugs, on human lung cancer cell lines. Int J Cancer 1990; 46: 326–9.
8. Carney DN, Gazdar AF, Bepler G, et al. Establishment and identification of small cell cancer cell lines having classic and variant features. Cancer Res 1985; 45: 2913–23.
9. Bergh J, Larsson E, Zech L, Nilsson K. Establishment and characterization of two neoplastic cell lines (U-1285 and U-1568) derived from small cell carcinoma of the lung. Acta Pathol Microbiol Immun Scand Sect A 1982; 90: 149–58.
10. Bergh J, Nilsson K, Ekman R, Giovanella B. Establishment and characterization of cell lines from human small cell and large cell carcinomas of the lung. Acta Pathol Microbiol Immun Scand Sect A 1985; 93: 133–47.
11. Bergh J, Nilsson K, Zech L, Giovanella B. Establishment and characterization of a continuous lung squamous cell carcinoma cell line. Anticancer Res 1981; 1: 317–22.
12. de Leij L, Poppema S, Nulend JK, et al. Neuroendocrine differentiation antigen on human lung carcinoma and Kulchitski cells. Cancer Res 1985; 45: 2192–200.
13. Okabe T, Kaizu T, Ozawa K, Urabe A, Takaku F. Elimination of small cell lung cancer cells in vitro from human bone marrow by a monoclonal antibody. Cancer Res 1985; 45: 1930–3.
14. Nilsson O, Brezicka FT, Holmgren J, et al. Detection of a ganglioside antigen associated with small cell lung carcinomas using monoclonal antibodies directed against fucosyl-G_{M1}. Cancer Res 1986; 46: 1403–7.
15. Sundström C, Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer 1976; 17: 565–77.
16. Larsson R, Nygren P: Pharmacological modification of multidrug resistance in vitro detected by a novel fluorometric microculture cytotoxicity assay. Resistance reversal and selective cytotoxic actions of cyclosporin A and verapamil on MDR leukemia T-cells. Int J Cancer 1990; 46: 67–73.
17. Larsson R, Nygren P, Ekberg M, Slater L. Chemotherapeutic drug sensitivity testing of human leukemia cells using a semiautomated fluorometric assay. Leukemia 1990; 4 567–71.
18. Larsson R, Kristenssen J, Sandberg C, Nygren P. Laboratory determination of chemotherapeutic drug resistance in tumor cells from patients with leukemia using a fluorometric microculture cytotoxicity assay. Int J Cancer 1992; 50: 177–85.
19. Mogensen KE, Bandu M-T, Vignaux F, Aguet M, Gresser I. Binding of [^{125}I]-labeled human alpha interferon to human lymphoid cells. Int J Cancer 1981; 28: 575–85.
20. Kartner N, Evernden-Porelle D, Bradley G, Ling V. Detection of P-glycoprotein in multidrug resistant cell lines by monoclonal antibodies. Nature 1989; 316: 820–3.
21. Dubois M-F, Ferrieux C, Lebon P. Synergistic cytotoxic effects of recombinant human tumor necrosis factor, interferons and heat-stress. Cancer Res 1989; 49: 5618–22.
22. Mattson K, Niiranen A, Holsti L, Pyrhönen S, Cantell K. Low dose of natural alpha interferon as maintenance therapy for small cell lung cancer. A phase III study. (Abstract) Proc Am Soc Clin Oncol 1989; 8: 227.
23. Carmichael J, Mitchell JB, DeGraff WG, et al. Chemosensitivity testing of human lung cancer cell lines using the MTT assay. Br J Cancer 1985; 57: 540–7.
24. Nygren P, Fridborg H, Csoka K, et al. Detection of tumor-specific cytotoxic drug activity in vitro using the fluorometric microculture cytotoxicity assay and primary cultures of tumor cells from patients. Int J Cancer 1994; 56: 715–20.
25. Carney DN, Berendsen H, Bergh J, et al. Biological response modifiers in the management of small cell lung cancer: a consensus report. Lung Cancer 1989; 5: 143–5.
26. Ucer U, Bartsch H, Scheurich P, Pfizenmainer K. Biological effects of gamma-interferon on human tumor cells: Quantity and affinity of cell membrane receptors for gamma-IFN in relation to growth inhibition and induction of HLA-DR expression. Int J Cancer 1985; 36: 103–8.
27. Tovey MG, Dron M, Mogensen KE, et al. Isolation of Daudi cells with reduced sensitivity to interferon: II. On the mechanism of resistance. J Gen Virol 1983; 64: 2449–53.
28. Pfeffer LM, Stebbing N, Donner DB. Cytoskeletal association of human alpha-interferon-receptor complexes in interferon-sensitive and resistant lymphoblastoid cells. Proc Natl Acad Sci USA 1987; 84: 3249–53.
29. Branca AA, Baglioni C. Evidence that type I and II IFNs have different receptors. Nature 1981; 294: 768–70.
30. Hovanessian AG, Wood JN. Anticellular and antiviral effects of pppA(2′p5′A)n. Virology 1980; 101: 81–90.
31. Krishnan I, Baglioni C. Elevated levels of (2′-5′)oligoadenylic acid polymerase activity in growth-arrested human lymphoblastoid Namalva cells. Mol Cell Biol 1981; 1: 932–8.
32. Fish EN, Banerjee K, Stebbing N. The role of three domains in the biological activity of human interferon-α. J Interferon Res 1989; 9: 97–114.