Eur J Cancer, Vol. 29A, No. 2, pp. 238-241, 1993.
Printed in Great Britain

0964-1947/93 $5.00 + 0.00
© 1992 Pergamon Press Ltd

MHC Class I Expression on Prelymphomatous and Lymphomatous B-cells is not Inhibited by an Eμ-*myc* Transgene

Ignacio Algarra, Santiago Silva and Hans-Gustaf Ljunggren

Increased expression of oncogenes from the *myc* family has been associated with down-regulation of major histocompatibility complex (MHC) class I molecules. In certain models this has been suggested to contribute to tumour progression. Transgenic mice bearing the cellular *myc* oncogene coupled to the lymphoid-specific immunoglobulin heavy chain enhancer (Eμ) develop clonal B lymphoid malignancies early in life. We have asked if expression of such a constitutively activated Eμ-*myc* transgene in BALB/c mice affects MHC class I expression. H-2K^d and D^d expression on prelymphomatous and lymphomatous B-cells as well as newly established pre-B or B lymphoma cell lines derived from Eμ-*myc* transgenic BALB/c mice were analysed. The results reveal no down-regulated or otherwise altered expression of H-2K^d or D^d on any of the cell populations examined. The results are discussed in relation to the *myc* associated down-regulation of MHC class I molecules observed in other experimental models.
Eur J Cancer, Vol. 29A, No. 2, pp. 238–241, 1993.

INTRODUCTION

THE PROTEINS encoded by the *myc* gene family are involved in the control of cell proliferation and differentiation [1]. Altered c-*myc* expression has been strongly implicated in neoplasia and c-*myc* is activated in many small cell lung carcinomas, mammary carcinomas, colon carcinomas, cervical carcinomas, melanomas and lymphoid tumours. In most Burkitt lymphomas and murine plasmacytomas, the *myc* gene has been activated by translocation to the immunoglobulin heavy (IgH)-chain locus [2, 3].

The effects of activated oncogenes on cells in their natural environment can be assessed through the use of transgenic mice [4]. Eμ-*myc* transgenic mice have been generated by introducing a DNA sequence designated Eμ-*myc* isolated from a mouse plasmacytoma in which a normal *myc* gene had become coupled to the IgH-chain enhancer [5]. The transgene is expressd in B lymphoid cells in all lymphoid tissues analysed with the highest levels detected in the spleen and lymph nodes [6]. The Eμ-*myc* transgenic mice almost invariably develop B lymphoid malignancies within a few months after birth and have served as a model to study the incidence, pathology and cell surface phenotype of malignant lymphomas of B-cell lineage [5–10].

Cell surface glycoproteins encoded by the major histocompatibility complex (MHC) are of crucial importance in cell mediated immunity. Cytotoxic T-cells (CTL) can recognise antigens only when these are presented as short peptides in association with the class I molecules of the MHC [11]. Therefore, tumour cells lacking MHC class I expression will not be subject to CTL-mediated lysis. MHC class I expression has been shown to be down-modulated by high expression of the c-*myc* or N-*myc* oncogenes in certain tumour models and it has been suggested that this may contribute to tumour progression [12–14]. These studies led us to analyse the effect of a constitutively activated c-*myc* transgene on MHC class I expression on premalignant and malignant cells of B cell origin derived from Eμ-*myc* transgenic mice of BALB/c background.

Correspondence to H.-G. Ljunggren.
S. Silva and H.-G. Ljunggren are at the Department of Tumor Biology, Karolinska Institutet, Box 60 400, S-104 01 Stockholm, Sweden; and I. Algarra is at the Servicio de Analisis Clinicos e Inmunologia, Hospital Virgen de las Nieves, Universidad de Granada, 18014 Granada, Spain.
Revised 17 July 1992; accepted 6 Aug. 1992.

MATERIALS AND METHODS

Mice

(C57BL/6XSJL)F_2-Eμ-*myc* transgenic mice were kindly supplied by Dr A.W. Harris. The transgene was introduced into the BALB/c strain by seven repeated back-crosses of Eμ-*myc* carriers with normal BALB/c mice. Weanling mice were screened for the presence of the Eμ-*myc* transgene by polymerase chain reaction (PCR). DNA was prepared either from blood lysates or from the tails and hybridised with a OX-174 probe.

Antibodies and immunofluorescence analysis

For immunofluorescence studies, splenocytes were depleted of erythrocytes by osmotic shock [vortexed 10 sec in 4 ml water prior to addition of 400 μl 10X phosphate buffered saline (PBS)]. Subsequently, 10^6 cells were incubated with 100 μl anti-mouse Ig monoclonal antibodies (mabs) (diluted 1:50) in PBS for 30 min on ice, washed twice with PBS, and incubated for an additional 30 min on ice with 100 μl anti-class I mabs (anti-K^d mabs diluted 1:40, anti-D^d mabs diluted 1:30), washed in PBS, and fixed with 1% formaldehyde. Single and dual colour immunofluorescence analysis was performed on a FACS 4 cell sorter and on FACScan cell analyser (Becton and Dickinson, Mountain View, California, USA). The present order of labelling prevented the anti-mouse Ig mabs to bind to the murine anti-class I mabs. Cells not treated with antibodies were used as negative controls. Affinity purified phycoerythrin (red) conjugated goat anti-mouse Ig antibodies were obtained from Southern Biotechnology Associates (Birmingham, Alabama, USA). Fluorescein isothiocyanate (FITC, green) conjugated mouse anti-H-2K^d (SF1-1.1) and anti-H-2D^d (34-5-8) mabs were obtained from Scan Biotech (Falkenberg, Sweden). All mabs

were stored dark in solution at 4°C at a concentration of 1.0 mg/ml.

Tumour cell lines

Tumour cells were adapted from Eμ-*myc* transgenic mice and adapted to *in vitro* growth in RPMI 1640 tissue culture medium supplemented with 10% fetal calf serum and antibiotics. The classification as B-cell or preB-cell lymphoma was based on the Ig^+ or Ig^- cell surface (s) phenotype, respectively.

RESULTS AND DISCUSSION

Descendants of Eμ-*myc* transgenic mice of BALB/c background were monitored for spontaneous tumour development. Approximately 50% of the mice developed fatal lymphomas within 10–20 weeks of age. The latency periods and tumour incidence were similar to the results reported by Harris *et al.* [9]. Tumours generally invaded the mesenteric lymph nodes and paravertebral lymph nodes, spleen, cervical lymph nodes and, occasionally, the thymus and the intracranial cavity.

Prelymphomatous 5-week-old Eμ-*myc*$^+$ mice of BALB/c background express reduced numbers of surface immunoglobulin positive (sIg^+) cells in the spleen. This is most likely due to an abnormally expanded, but non-malignant, population of sIg^- B-cell precursors [7]. Single colour immunofluorescence analysis of H-2K^d and D^d expression on non-purified spleen cells from such mice, including the substantial pool of sIg^- B-cells, revealed no detectable difference in either H-2K^d or D^d expression when compared with spleen cells from Eμ-*myc*$^-$ backcross or normal BALB/c mice (Fig. 1a). Dual colour immunofluorescence analysis confirmed that sIg^+ cells isolated from the prelymphomatous 5-week-old Eμ-*myc*$^+$ mice were included in the MHC class I positive population; H-2K^d as well as D^d levels did not differ from those on sIg^+ cells isolated from Eμ-*myc*$^-$ backcross or normal BALB/c control mice (Fig. 1b). Analysis of H-2K^d and D^d expression on sIg^+ lymphomatous splenocytes from 3-month-old Eμ-*myc*$^+$ mice revealed identical results; no downregulation or otherwise altered expression of H-2K^d or D^d as compared with similar fractions of cells from normal BALB/c control mice (data not shown). In all experiments performed, fluorescence activated cell sorter curves as well as actual numbers of positive cells were compared. In the latter cases, no significant differences were noted supporting the image of the curves (data not shown).

A majority of the clonally distributed lymphomas in Eμ-*myc* transgenic mice have been identified as preB lymphomas or mixtures of preB and B lymphomas while some 19% represent true B-cell lymphomas [9]. We established *in vitro* lines of lymphomas from mice which had developed peripheral lymphomas. H-2K^d and D^d expression was analysed on one sIg^+ and one sIg^- line after 3–5 weeks in culture; the intracranially derived B-cell lymphoma line (Eμ-*myc*C-680-8/1) and the submaxillary derived preB-cell lymphoma line (Eμ-*myc*C-680-8/2). Both tumours readily expressed clearly detectable levels of H-2K^d and D^d at the cell surface and gave a similar pattern of reactivity when compared against each other (Fig. 2; Eμ-*myc*C-680-8/2 not shown). Consistent with the H-2K^d and D^d positive phenotype of Eμ-*myc*C-680-8/1, the line was also sensitive to anti-H-2^d specific CTL lysis (data not shown).

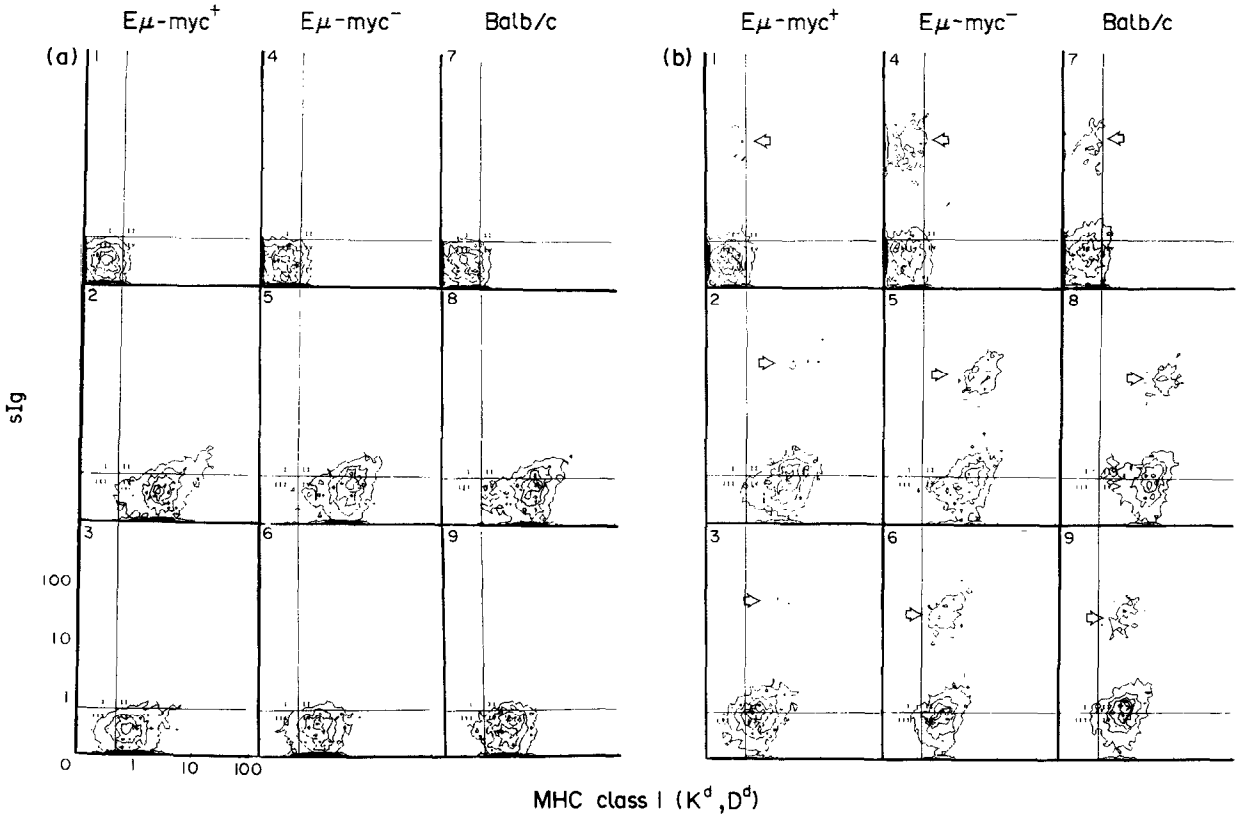

Fig. 1. Single and dual colour immunofluorescence analysis of Ig (y-axis) and H-2K^d/D^d (x-axis) expression on splenocytes from 5-week-old prelymphomatous Eμ-myc$^+$ [1–3], Eμ-*myc*$^-$ [4–6] and BALB/c [7–9] mice. (a) Single colour immunofluorescence. 1,4,7, unstained controls; 2,5,8,K^d expression; 3,6,9,D^d expression. (b) Dual colour immunofluorescence. 1,4,7,Ig expression; 2,5,8,Ig and K^d expression; 3,6,9,Ig and D^d expression. Arrows indicate H-2K^d/D^d expression on Ig-positive fractions.

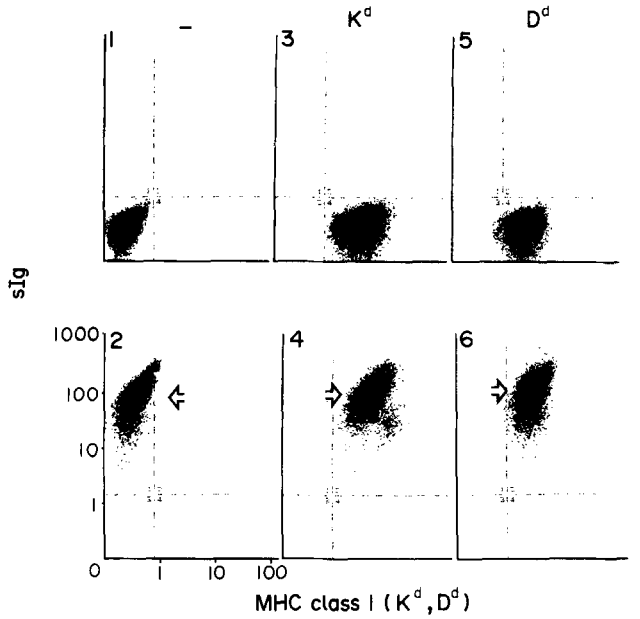

Fig. 2. Dual colour immnofluorescence analysis of Ig (y-axis) and H-2K^d/D^d (x-axis) on an intracranial B lymphoma adapted to tissue culture. 1, Negative control; 3, K^d expression; 5, D^d expression; 2, Ig expression; 4, Ig and K^d expression; 6, Ig and D^d expression. Arrows indicate H-2K^{d}D^d expression on Ig-positive fractions.

Taken together these data suggest that expression of MHC class I molecules on splenocytes derived from mice carrying a constitutively activated Eμ-*myc* transgene is not significantly altered compared with expression on splenocytes from normal BALB/c mice or Eμ-*myc*$^-$ backcross mice.

The relationship between expression of the N-*myc* or c-*myc* oncogenes and MHC class I molecules has been investigated in several studies. Two transfection models based on a rat neuroblastoma cell line and human melanomas are frequently cited [12, 13]. These studies have convincingly revealed an association between amplified N-*myc* and c-*myc* expression, respectively, and down-regulation of MHC class I expression. In contrast to these results, it was demonstrated that expression of N-*myc* in rat fibroblasts or other human neuroblastoma cell lines was independent of MHC class I expression [12, 15]. Further, enhancement of tumorigenicity caused by elevated c-*myc* expression did not correlate with MHC class I expression in the murine polyoma virus-induced SEWA tumour [16]. Nor could any correlation be observed when HLA class I expression and c-*myc* expression were compared in human non-small cell lung carcinomas [17].

It is not clear why increased c-*myc* expression alters MHC class I expression in certain cells but not in others. One important factor determining the effects on class I expression may be the c-*myc* levels expressed. Eμ-*myc* RNA in the preneoplastic transgenic spleen is about five-fold more abundant than endogenous c-*myc* transcripts in a normal spleen and resembles that of c-*myc* RNA levels seen in splenocytes 24 h after stimulation with the B-cell mitogen lipopolysaccaride or the T-cell mitogen concavalin A [6]. The *myc* RNA levels in prelymphomatous splenocytes were also comparable to that in Eμ-*myc* tumour cell lines or in conventional plasmacytomas. No differences in expression of the Eμ-*myc* transgene were detected when B lymphomas were compared with preB lymphomas [6]. In certain cases, the discrepancy may also be explained by the histological cell type or analysed or species studied. While N-*myc* down-regulated class I expression was seen in rat neuroblastomas, no effect was seen when the same N-*myc* gene was expressed in rat fibroblasts [12] or when N-*myc* was transfected into human neuroblastoma cell lines [15]. Further, only some MHC class I locus products may be affected by over-expression of the *myc* gene products. In the case of human melanoma, c-*myc* preferentially suppresses the HLA B-locus and possibly the C-locus products. The A-locus encoded class I molecules do not stay completely unaffected, but their suppression is limited [18].

Taken together, it is clear that members of the *myc* gene family under certain circumstances may affect MHC class I expression. In one study, down-regulation of class I expression was demonstrated to occur through enhancer inactivation [19]. Yet, in other models the mechanism between *myc* mediated down-regulation of class I expression is less clear. Down-regulation of MHC class I expression may alter susceptibility to CTL lysis and favour tumour progression. However, in other models including the present study, the first to our knowledge based on a transgenic mouse system, there is no obvious association between activated or amplified *myc* genes and MHC class I gene expression. Further studies will be needed to explore the detailed relationship between MHC class I and *myc* expression.

1. Luscher B, Eisenman RN. New light on *myc* and *myb*. *Genes Dev* 1990, **4**, 2025–2035.
2. Klein G, Klein E. *myc*/Ig juxtaposition by chromosomal translocations: some new insights, puzzles and paradoxes. *Immunol Today* 1985, **6**, 208–215.
3. Cory S. Activation of cellular oncogenes in hemopoetic cells by chromosome translocation. *Adv Cancer Res* 1986, **47**, 189–234.
4. Hanahan D. Transgenic mice as probes into complex systems. *Science* 1989, **246**, 1265–1275.
5. Adams JM, Harris AW, Pinhert CA, *et al.* The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature* 1985, **318**, 533–538.
6. Alexander WS, Schrader JW, Adams JM. Expression of the c-*myc* oncogene under control of an immunoglobulin enhancer in Eμ-*myc* transgeneic mice. *Mol Cell Biol* 1987, **7**, 1436–1444.
7. Langdon WY, Harris AW, Cory S, Adams JM. The c-*myc* oncogene perturbs B lymphocyte development in Eμ-*myc* transgenic mice. *Cell* 1986, **47**, 11–18.
8. Langdon WY, Harris AW, Cory S. Growth of Eμ-*myc* transgenic B lymphoid cells *in vitro* and their evolution towards autonomy. *Oncogene Res* 1988, **3**, 271–279.
9. Harris AW, Pinkert CA, Crawford M,Langdon WY, Brinster RL, Adams JM. The Eμ-*myc* transgenic mouse. A model for high-incidence spontaneous lymphoma and leukemia of early B cells. *J Exp Med* 1988, **167**, 353–371.
10. Sugiyama H, Silva S, Wang Y, *et al.* Abelson murine leukemia virus transforms preneoplastic Eμ-*myc* transgene carrying cells of the B lymphocyte lineage into plasmoblastic tumors. *Int J Cancer* 1990, **46**, 845–852.
11. Townsend A, Bodmer H. Antigen recognition by class I restricted T lymphocytes. *Ann Rev Immunol* 1989, **7**, 601–624.
12. Bernards R, Dessain SK, Weinberg RA. N-*myc* amplification causes down-modulation of MHC class I antigen expression in neuroblastoma. *Cell* 1986, **47**, 667–674.
13. Versteeg R, Noordermeer IA, Kruse-Wolters KM, Ruiter DJ, Schrier PI. C-*myc* down regulates class I HLA expression in human melanomas. *EMBO J* 1988, **7**, 1023–1029.
14. Gaforio JJ, Perez M, Algarra I, Mialdea MJ, Ljunggren HG, Garrido F. Differential mRNA levels of c-*myc*, c-*fos* and MHC class I in several tumor clones of a murine fibrosarcoma. *Int J Cancer* 1991, **49**, 906–910.
15. Feltner DE, Cooper M, Weber J, Isreal MA, Thiele CJ. Expression of class I histocompatibility antigens in neuroectodermal tumors is independent of the expression of a transfected neuroblastoma *myc* gene. *J Immunol* 1989, **143**, 4292–4299.
16. Dahllöf B. Down regulation of MHC class I antigens is not a general mechanism for the increased tumorigenicity caused by c-*myc* amplification. *Oncogene* 1990, **5**, 433–435.

17. Redondo M, Ruiz-Cabello F, Concha A, *et al.* Altered HLA class I expression in non-small cell lung cancer is independent of c-*myc* activation. *Cancer Res* 1991, **51**, 4948–4954.
18. Versteeg R, Kruse-Wolters KM, Plomp AC, *et al.* Suppression of class I human leukocyte antigen by c-*myc* is locus specific. *J Exp Med* 1989, **170**, 621–635.
19. Leonardo M, Rustigi AK, Schievella AR, Bernards R. Suppression of MHC class I gene expression by N-*myc* through enhancer activation. *EMBO J* 1989, **8**, 3351–3355.

Acknowledgements—We thank Drs C. Öhlén, P. Höglund, K. Kärre, G. Klein and F. Garrido for fruitful discussions and B. Brise for help with FACS analysis.

This work was supported by grants from the U.S. Public Health Service Grants 5 RO1 CA-25250-06 and 1 RO1 CA-44882-01 awarded by the National Cancer Society, The Swedish Society for Medicine, The Swedish Cancer Foundation, The Swedish Medical Research Council and Associazione Malattie Rare "Mauro Baschirotto". Dr I. Algarra was the recipient of a scholarship from the Swedish Institute.

Eur J Cancer, Vol. 29A, No. 2, pp. 241–245, 1993.
Printed in Great Britain

0964–1947/93 $5.00 + 0.00
© 1992 Pergamon Press Ltd

Insulin-like Growth Factor-I-dependent Growth and *in vitro* Chemosensitivity of Ewing's Sarcoma and Peripheral Primitive Neuroectodermal Tumour Cell Lines

Susanne Hofbauer, Gerhard Hamilton, Gerhard Theyer, Karin Wollmann and Franz Gabor

Serum-free growth of Ewing's sarcoma (ES) and primitive peripheral neuroectodermal tumour (pPNET) cell lines was achieved by supplementing a basal medium with insulin-like growth factor-I (IGF-I). These cultures were used to investigate the sensitivity of 3 ES (EW-2, RD-ES, SK-ES-1) and 3 pPNET (SIM-1, KAL, SAL) cell lines to a panel of anti-tumour agents in short-term (48-h) proliferation assays. Of the four cytostatic drugs included in the currently used multi-drug regimens, cyclophosphamide, doxorubicin and actinomycin-D inhibit the proliferation of the cell lines with high efficacy, whereas the vinca alkaloids were less effective. Cisplatin, etoposide, mitomycin-C and mitoxanthrone were also found to have a high inhibitory activity in this *in vitro* ES/pPNET system. The most remarkable effect was observed for cytosine arabinoside (ARA-C), which gave a half-maximal inhibition at drug concentrations approximately 5000 times below the clinical peak plasma concentrations (250 μg/ml). The ARA-C sensitivity of ES and pPNET cell lines is comparable with the established ARA-C sensitivities of leukaemia-derived cells. The different ES and pPNET cell lines showed a rather uniform response to the different cytostatic drugs with decreased sensitivity of individual pPNET cell lines to vinblastin, ARA-C and mitoxanthrone. Modulation of the IGF-I/IGF-I receptor/IGF-I binding protein system, which seems to constitute an important stimulator of cell growth in neuroectoderm-derived or -related tumours, can be used to enhance the drug sensitivity of the tumour cells *in vivo* or in *in vitro* therapeutic procedures. According to our results, serum-free conditions for autologous bone marrow purification are expected to result in significantly increased chemosensitivity of ES and pPNET cells in response to anthracyclines and cisplatin.
Eur J Cancer, Vol. 29A, No. 2, pp. 241–245, 1993.

INTRODUCTION

EWING'S SARCOMA (ES), the second most frequent bone tumour in childhood, represents a undifferentiated small round-cell tumour of uncertain origin, expressing neuroectodermal markers and a unique t(11;22) (q24;q12) reciprocal translocation or a deletion of the long arm of chromosome 22, del(22) (q12) [1]. Recently, with the help of the HBA-71 monoclonal antibody [2], high levels of MIC2, a pseudoautosomal human gene product involved in T-lymphocyte cell adhesion, has been found in ES and primitive peripheral neuroectodermal tumours (pPNET) [3]. The ES-related peripheral (extracranial) pPNET are small round-cell malignancies of presumed neural crest origin, which share, among other characteristics, the chromosomal reciprocal translocation t(11;22) and the expression of high levels of MIC2/HBA-71-antigen with ES [3, 4]. The HBA-71-antibody inhibits the growth of ES and pPNET cell lines by interfering with the action of insulin-like growth factor-I (IGF-I), which seems to function as specific growth factor for both tumours [5]. The recognition of pPNET as a distinct entity is based on

Correspondence to G. Hamilton.
S. Hofbauer and G. Hamilton are at the Department of Surgery; Gerhard Theyer is at the Department of Urology, University of Vienna, Alserstraße 4, A-1090 Vienna; and Karin Wollmann and Franz Gabor are at the Institute of Pharmaceutical Technology, University of Vienna, Waehringerstrasse 25, A-1090 Vienna, Austria.
Revised 14 May 1992; accepted 20 July 1992.