Virchows Arch (1997) 430:41–46

ORIGINAL ARTICLE

Tetsuro Sugimoto · Akihiro Umezawa · Jun-ichi Hata

Neurogenic potential of Ewing's sarcoma cells

Received: 2 July 1996 / Accepted 2 August 1996

Abstract We investigated the capacity of eight well-characterized Ewing's sarcoma cell lines to differentiate towards a neural phenotype. Ewing's sarcoma cells expressed the neuroectoderm-associated antigens such as nerve growth factor (NGF) receptor, N-CAM (6H7 and Leu-19) and Leu-7. Ewing's sarcoma cells also exhibited the potential for neural differentiation at the mRNA level; neuron-specific medium- and low-sized filament (NF-M and NF-L) expression was induced by dibutyryladenosine cyclic monophosphate. The pattern of expression of NF-L obtained by using alternative polyadenylation sites in Ewing's sarcoma cells differed from that in peripheral primitive neuroectodermal tumour (PNET) cells, and was similar to that in undifferentiated neural tissues. Furthermore, the NGF receptors detected by immunohistochemistry were found to be non-functional as assayed by *c-fos* induction with NGF treatment. The results indicate that Ewing's sarcoma cells maintain a primitive phenotype and have the potential to differentiate into a neural phenotype, indicating that Ewing's sarcoma is distinct from PNET.

Key words Ewing's sarcoma · Neural differentiation · Neurofilament protein · Nerve growth factor receptor

Introduction

Ewing's sarcoma is a rare, small round-cell undifferentiated tumour of bone and soft tissues [13]. The histogenesis of Ewing's sarcoma remains obscure because of the lack of unequivocal lineage markers, and its origin has been debated ever since Ewing first described the disease as a diffuse endothelioma of bone [14]. A large number of studies have indicated that Ewing's sarcoma have neural traits [2, 28, 32–34, 48]. Certain populations of Ewing's sarcoma have been shown to be positive immunohistochemically for neuron specific enolase (NSE) and Leu-7 [2, 48] and were found by electron microscopy to contain neurosecretory granules [2]. Experimentally, it was found that cultured Ewing's sarcoma cell line showed neural characteristics after treatment to induce differentiation [8, 18, 40, 41].

Ewing's sarcoma has also been shown to possess specific chromosomal translocations [26, 52, 55, 57]. These features are shared with peripheral primitive neuroectodermal tumour (PNET). Ewing's sarcoma and PNET tumours have a similar morphology, originate in the soft tissue and bone, and show similar clinical features. Analysis of the chimeric genes revealed that Ewing's sarcoma/PNET have the same fusion transcripts and belong to a common entity [11]. In certain cases, a transitional morphology between Ewing's sarcoma and PNET was observed [25]. Thus, clinical, morphological, immunohistochemical and genetic analyses link Ewing's sarcoma to PNET.

Although the biological and morphological characteristics of Ewing's sarcoma are very similar to those of PNET, a relationship between the two tumours has been questioned [9, 20, 46, 56]. Ewing's sarcoma may also be classified as a neuroectodermal tumour if it is shown to have the potential for neural differentiation. Jaffe et al. [25] found tumours with neuroectodermal characteristics in Ewing's sarcoma of bone and termed them "neuroectodermal tumour of bone" and in this way the distinction between Ewing's sarcoma and PNET has become blurred [36]. According to Marina's criteria [35], PNET is a tumour with any neural characteristic. In this study, we investigated the capacity for differentiation towards a neural phenotype of various Ewing's sarcoma cell lines. Our results indicate that Ewing's sarcoma has the potential for neural differentiation at the mRNA level and originates from less differentiated neural tissues than PNET.

T. Sugimoto · A. Umezawa · J. Hata (✉)
Department of Pathology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan
Tel.: (81)3-3353-1211, ext. 2675, Fax: (81) 3-3353-3290

Materials and methods

Eight Ewing's sarcoma cell lines were used in this study. NCR-EW1, EW2, EW3, EW4 [19], SCCH-196 [22] and W-ES [15] have been maintained in our laboratory. Those cell lines were established from Ewing's sarcoma that reacted with the specific antibody 5C11 and did not have any neural characteristic detectable immunohistochemically or electron-microscopically. The antibody 5C11 against the cell surface antigen of Ewing's sarcoma has been generated and its specificity described previously [19]. Briefly, 5C11 specifically reacted with Ewing's sarcoma but not with other small round cell tumours in childhood including neuroblastoma, rhabdomyosarcoma and malignant lymphoma. RD-ES and SK-ES1 [6] were purchased from the American Type Culture Collection (ATCC, Rockville, Md., USA). The typical chromosomal abnormality, t(11;22)(q24;q12), was observed in NCR-EW2, SCCH-196 and W-ES. NCR-EW1 lost the reactivity for 5C11 during culture [19].

The cells were cultured at 37°C in Dulbecco's modified minimum essential medium (Gibco) in the presence of 10% fetal bovine serum (Gibco) and 5% carbon dioxide in the 25-cm^2 culture flasks. The cells were seeded in 0.05 M EDTA in phosphate buffered saline without calcium and magnesium. Ewing's sarcoma cell lines were differentiated with N_6-O_2-dibutyryladenosine-3;5′-cyclic monophosphate (db-cAMP, Sigma). At the concentrations of 0.5 to 2.5 mM db-cAMP was added to the medium. The medium was changed every third day and culturing was maintained for up to 12 days. The PNET cell line NCR-PN1 and the neuroblastoma cell line NCR-NB3 [19] served as controls with the same treatments.

Cultures were examined for neuroectoderm-associated antigens by immunocytochemistry of the cytospin samples and by flowcytometry (Epics-Profile, Coulter). The primary antibodies used were as follows: Ewing's sarcoma specific antibody 5C11 [19], antibodies against the neurofilament protein 200 kDa (NF-H, Labsystems), 160 kDa (NF-M, Boehringer Mannheim) and 68 kDa (NF-L, Dako), 6H7 [42] and Leu-19 (Becton Dickinson) directed against a neural-cell adhesion molecule (N-CAM), nerve growth factor (NGF)-receptor (Boehringer Mannheim), Leu-7 (Becton Dickinson), choline acetyltransferase (CAT, Chemicon), tyrosine hydroxylase (TH; Chemicon), NSE (Dako) and chromogranin A (Dako). Horseradish peroxidase-labelled anti-mouse or anti-rabbit immunoglobulin antibody and fluorescein isothiocyanate-labelled anti-mouse immunoglobulin antibody (Dako) were used for the secondary antibodies.

RNA blot analysis was performed as described previously [53, 54]. For the RNA extraction, the cells were homogenized in guanidine-isothiocyanate solution and then centrifuged on cesium chloride. Five micrograms of RNA was electrophoresed in 1% agarose gel and transferred to a nylon filter (Gene Screen Plus, DuPont Company NEN Products). Almost equal amounts of rRNA were recovered from the cells, which was determined by methylene blue staining. The filter was treated for 30 min in a solution containing 5× SSPE (0.9 M NaCl, 50 mM NaH_2PO_4, 5 mM EDTA), 5× Denhardt's solution, 1% sodium dodecyl sulphate (SDS) and 0.01% poly A (Boehringer Mannheim). The DNA probes were prepared with [a-^{32}P] dCTP (3000 Ci/mmole, Amersham) by the random primer method. Hybridization was performed at 60°C for 16 h. The hybridized filter was washed in 2× SSC (1× SSC = 0.15 M NaCl, 50 mM NaH_2PO_4, 5 mM EDTA) at room temperature and 65°C, respectively. Then it was washed in 0.1% SDS and 0.1× SSC at 65°C. The filter was exposed to radiography film at –80°C. An actin cDNA probe [23] served as the control.

Ewing's sarcoma cell lines, which are positive for the NGF receptor, were examined for the early response gene after NGF stimulation (50 ng/ml). A cDNA probe for *c-fos*, NF-L (1.4 kb, Eco RI fragment, NF5.1.) and NF-M (1.2 kb, Eco RI fragment, NF1.2.) [38] obtained from ATCC was used. The rat phaeochromocytoma cell line PC-12 served as the control.

Results

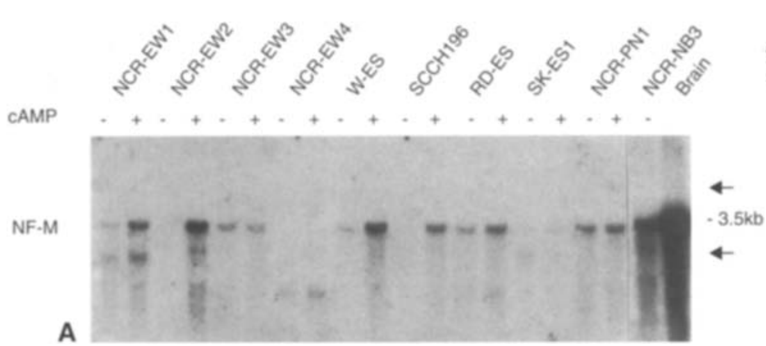
Results of the immunocytochemical examination for the neuroectoderm-associated antigens in Ewing's sarcoma are summarized in Table 1. NGF receptor, N-CAM (6H7 and Leu-19), NSE and Leu-7 showed positive reactions in all cell lines except NCR-EW1 and NCR-EW4 by the flowcytometry analysis. CAT showed positive reactions in NCR-EW2 and W-ES. TH and chromogranin A were negative in all the cell lines. NF-M or -H expression was observed only in two cell lines, W-ES and NCR-EW4, before db-cAMP treatment. However, after db-cAMP treatment, positive expressions were observed in all cell lines examined. No NF-L expression was observed in any cell lines before or after db-cAMP treatment.

Certain Ewing's sarcoma cell lines reacted positively with NF-M in the immunocytochemical analysis. In order to determine whether the neurofilament proteins are regulated at the mRNA level, we performed RNA blot analysis. For a positive control, NF-M mRNA was detected at 3.5 kb in the brain and NCR-NB3. NF-M mRNA expression was observed only in both NCR-EW3 and W-ES cell lines before db-cAMP treatment. However, 12 days after db-cAMP treatment, all cell lines except NCR-EW4 expressed NF-M mRNA (Fig. 1A). The intensity and time-kinetics of NF-M induction by db-cAMP was different for each cell line. NF-M expression was detected one day after treatment and reached to a peak on day 6 in NCR-EW2 (Fig. 1B). In contrast, in SCCH196, the signal was very weak and was first detected 12 days after the treatment.

Similarly, NF-L mRNA was also increased after db-cAMP treatment. All cell lines except NCR-EW1 and NCR-EW4 expressed NF-L mRNA 12 days after the

Table 1. Expression of neuroectoderm-associated antigens in Ewing's sarcoma (–, Negative; +, positive; (+)*, positive after treatment with db-cAMP)

Cell line	NGF-R	N-CAM	Leu-7	NSE	CAT	NF-H	NF-M	NF-L
NCR-EW1	–	–	–	–	–	–	– (+)*	–
NCR-EW2	+	+	+	+	+	– (+)*	– (+)*	–
NCR-EW3	+	+	+	+	–	–	– (+)*	–
NCR-EW4	–	–	–	–	–	+	–	–
W-ES	+	+	+	+	+	+	+	–
SCCH196	+	+	+	+	–	–	– (+)*	–
RD-ES	+	+	+	+	–	– (+)*	– (+)*	–
SK-ES1	+	+	+	+	–	– (+)*	– (+)*	–



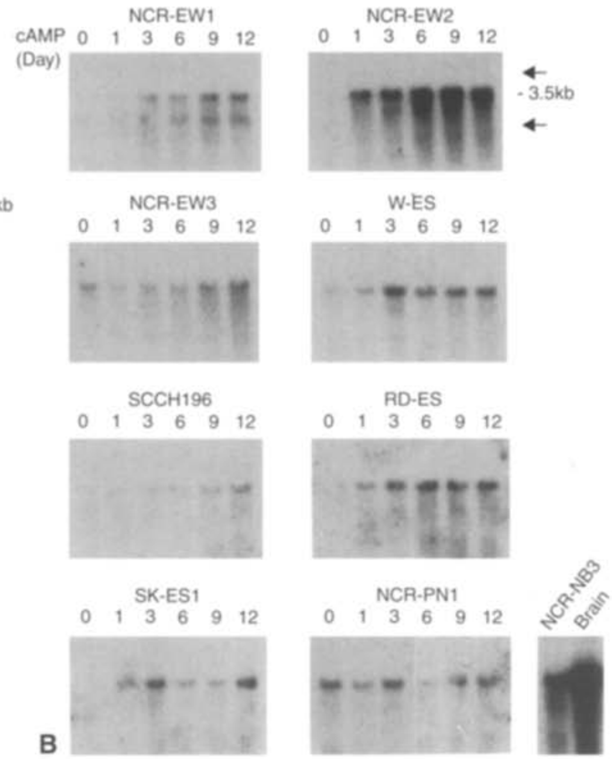
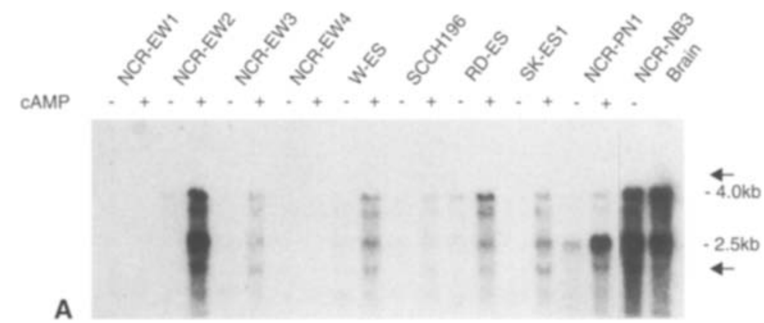


Fig. 1A, B NF-M expression in Ewing's sarcoma cells. **A** Most Ewing's sarcoma cell lines started to express NF-M after induction. Cells were induced to differentiate by treatment with db-cAMP for 12 days. The culture medium containing db-cAMP was changed every third day. Cells were either untreated (–) or treated with db-cAMP (+). **B** Time course of NF-M expression in NCR-EW1, NCR-EW2, NCR-EW3, W-ES, SCCH196, RD-ES, SK-ES1 and NCR-PN1 cells after treatment with db-cAMP for the number of days indicated. A PNET cell line, NCR-PN1; a neuroblastoma cell line, NCR-NB3; and brain served as the controls. Five micrograms of total RNA was electrophoresed in each lane. The blot was hybridized with the NF-M probe



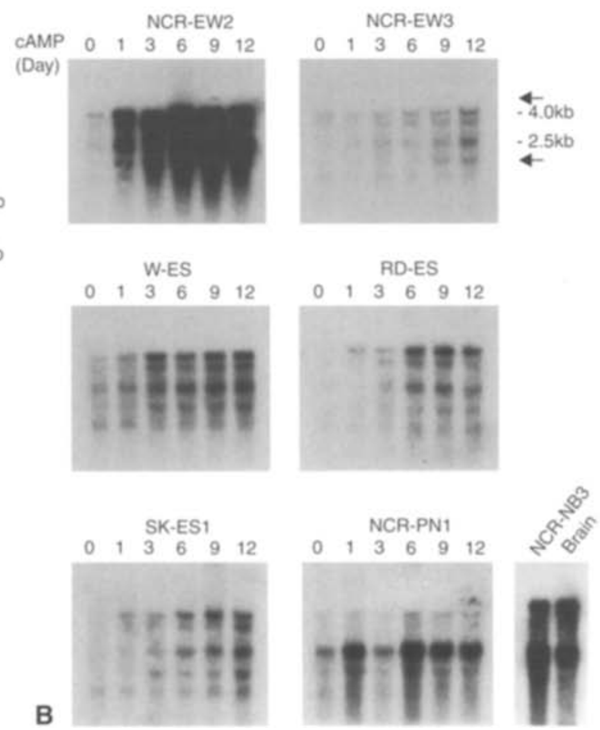


Fig. 2A, B NF-L expression in Ewing's sarcoma cells. **A** Cells were induced to differentiate by treatment with db-cAMP for 12 days. The culture medium containing db-cAMP was changed every third day. Cells were either untreated (–) or treated with db-cAMP (+). **B** Time course of NF-L expression in NCR-EW2, NCR-EW3, W-ES, RD-ES, SK-ES1 and NCR-PN1 cells after treatment with db-cAMP for the number of days indicated. A PNET cell line, NCR-PN1; a neuroblastoma cell line, NCR-NB3; and brain served as the controls. Five micrograms of total RNA was electrophoresed in each lane. The blot was hybridized with the NF-L probe

treatment (Fig. 2A). Although 2.5-kb NF-L mRNA was dominant in the NCR-PN1, NCR-NB3 and brain, the amounts of 2.5 and 4.0 kb mRNAs were approximately equal or 4.0 kb mRNA was even dominant in Ewing's sarcoma cell lines (Fig. 2A,B). The time-kinetics of NF-L induction showed that NF-L expression reached a peak on days 6–12 after exposure to db-cAMP and the ratio of the two transcripts did not change (Fig. 2B). These two different mRNAs showed the same pattern even after such a highly stringent washing condition as 0.05×SSC at 65°C for 30 min.

There are several reports on NGF receptor expression

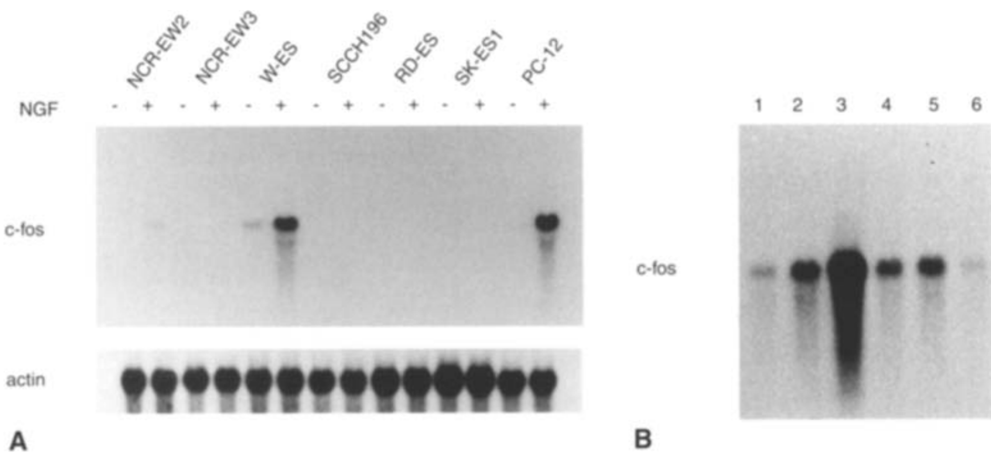


Fig. 3A, B Induction of *c-fos* mRNA by NGF in Ewing's sarcoma cells. **A** Cells were either untreated -(-) or treated with NGF (+) at a final concentration of 50 ng/ml for 30 min prior to extraction of RNA. A rat phaeochromocytoma cell line, PC-12, was served as the control. Five micrograms of total RNA was electrophoresed in each lane. The blot was hybridized with the *c-fos* probe (upper gel) first. Then, the blot was dehybridized and rehybridized with the actin probe (lower gel). **B** Time course of *c-fos* induction by NGF in W-ES cells. RNAs were extracted from the cells at 15 min (lane 2), 30 min (lane 3), 2 h (lane 4), 4 h (lane 5), 8 h (lane 6) after NGF (50 ng/ml) treatment. Lane 1 was the control (without NGF). Five micrograms of total RNA was electrophoresed in each lane. The blot was hybridized with the *c-fos* probe

in Ewing's sarcoma [17, 31]. However, these reports did not mention the biological importance of the receptor, including the presence of subtypes. PC12 cells, in which the NGF receptor is abundant, are known for strong transient transcription and a high mRNA level of *c-fos*. (Fig. 3A). This *c-fos* induction is well correlated with the location of the high-affinity receptor [2, 21] and we therefore used this system to determine whether the NGF receptor immunohistochemically detected on the Ewing's sarcoma cell lines is functional. Thirty minutes after NGF stimulation, *c-fos* expression was detected in only one cell line (W-ES) out of the six Ewing's sarcoma cell lines (Fig. 3A). The level of expression level of β-actin mRNA as the control remained unchanged after NGF stimulation. The time course in c-*fos* mRNA in W-ES cells demonstrated a typical transient expression (Fig. 3B).

Discussion

Cytogenetic analyses have indicated that Ewing's sarcoma and PNET have the same genetic aberration and belong to a common entity [11]. Ewing's sarcoma and PNET have specific chromosomal translocations [52, 57]. These translocations are represented by specific fusion transcripts: EWS/FLI-1 originating from a t(11; 22) translocation [4, 10, 43]; EWS/ERG originating from a t(21;22) translocation [44, 49]; EWS/ETV-1 originating from a t(7;22)(p22;q12) translocation [26]; and EWS/E1A-F [55]. In these fusion transcripts, the transcriptional activation domain of EWS is fused to FLI-1 or ERG and the transcripts are thought to be important in tumorigenesis. EWS/FLI-1 and EWS/ERG fusion transcripts were found in 95% of Ewing's sarcoma/PNET and were not correlated with phenotypic markers of differentiation such as neural protein expression [11, 12].

We have shown that Ewing's sarcoma cell lines do not have neural characteristics, but have the potential for neural differentiation. Ewing's sarcoma is thus distinct from the PNET which constitutively expresses neural phenotypic markers. Several differentiated stages of tumour cells have been described in different leukaemias [5]. Although Ewing's sarcoma and PNET probably arise from the same genetic aberration involving translocations between the EWS and ETS family genes, our results suggest that Ewing's sarcoma is derived from undifferentiated neural cells, is maintained in the undifferentiated state in vivo, and is therefore distinct from the PNET [1, 10]. These tumours probably represent different stages of differentiation in a tumour of common origin.

All eight Ewing's sarcoma cell lines examined expressed neurofilament (NF) mRNA, indicating that Ewing's sarcoma cells could differentiate to neural cells in vitro. In this study, we investigated the potential ability of Ewing's sarcoma cells to differentiate to a neural phenotype by using cell lines that reacted with the Ewing's sarcoma-specific antibody 5C11 but expressed no neural phenotypic markers. NFs, which are expressed in neurons and other cells of neuroectodermal origin, are composed of three groups of microheterogeneous subunits with molecular masses of 68 kDa (NF-L), 160 kDa (NF-M), and 200 kDa (NF-H) [50, 51]. NF expression is regulated in differentiating neural cells. NF-L and NF-M are expressed in the early stages of development and NF-H is expressed later [7, 47]. These attributes mainly to the differences in stage of neural differentiation.

There are two different NF-L transcripts, 4.0 kb and 2.5 kb in length [27, 30]. The expression of these mRNAs is controlled separately [24] and is regulated by alternative polyadenylation sites [39]. In Ewing's sarcoma cells, these two mRNAs were of equal abundance with the 4.0 kb form occasionally present at higher levels. In contrast, the 2.5-kb NF-L transcript was the major species in PNET cells. Differential transcript levels were probably the result of different polyadenylation sites rather than general degradation of RNA, since a similar pattern of ribosomal RNA was observed in both cells. Similar regulation by alternative polyadenylation is observed in the nerve specific genes N-CAM [3] and myelin proteolipid protein [16]. Although the biological activities of the alternatively polyadenylated mRNA is still not clear, the 2.5/4.0 kb ratio increased during the course of growth in nervous tissue [45]. The pattern of NF-L mRNA expression in Ewing's sarcoma cells may reflect the undifferentiated state of neural precursor cells, while the high levels of expression of the 2.5 kb transcript in PNET may reflect more differentiated neural cells.

Most Ewing's sarcoma cell lines express NGF receptor, but this receptor is non-functional. NGF is a neural peptide which plays important roles in differentiation, growth and maintenance of the sympathetic nerve cells and the sensory neural cells derived from the neural crest [29]. There are two types of receptors for NGF that differ in affinity, dissociation and molecular weight [37]. Only the high-affinity receptor, which is believed to consist of the low-affinity receptor and a 60-kDa peptide, appears to mediate the biological activity of NGF [17]. The lack of a functional NGF receptor may result either from the lack of high-affinity receptor or the lack of an intact signal pathway from the receptor to the nucleus.

A similar pattern of expression of neuroectoderm-associated antigens has suggested that Ewing's sarcoma is very similar to PNET. However, while Ewing's sarcoma cells showed a potential for neural differentiation, these cells had a less differentiated phenotype than PNET cells. The pattern of expression of NF-L transcripts suggested that Ewing's sarcoma may arise from neural precursor cells. Detailed analysis concerning the relationship between Ewing's sarcoma and PNET is required for further understanding of these closely related tumours.

Acknowledgements We wish to thank S. Kusakari, H. Suzuki, K. Takeichi, and H. Abe for their technical assistance. We dedicated this manuscript to Dr. T. Hirata, our colleague, who died of PNET in 1993. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and Cancer Research Grant from the Ministry of Health and Welfare, together with funds provided by the Entrustment of Research Program of the Foundation for Promotion of Cancer Research of Japan and by the Vehicle Racing Commemorative Foundation.

References

1. Bosselut RA, Zucman J, Cormier F, Delattre O, Roussel M, Thomas G, Ghysdael J (1994) DNA-binding and transcriptional activation properties of the EWS-FLI-1 fusion protein resulting from the t(11;22) translocation in Ewing sarcoma. Mol Cell Biol 14:3230–3241
2. Baker DL, Reddy DR, Pleasure D, Thorpe CL, Evans AE, Cohen PS, Ross AH (1989) Analysis of nerve growth factor receptor expression in human neuroblastoma and neuroepithelioma cell lines. Cancer Res 49:4142–4146
3. Barbas JA, Chaix JC, Steinmetz M, Goridis C (1988) Differential splicing and alternative polyadenylation generates distinct NCAM transcripts and proteins in the mouse. EMBO J 7:625–632
4. Ben-David Y, Giddens EB, Letwin K, Bernstein A (1991) Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the ets gene family, Fli-1, closely linked to c-ets-1. Genes Dev 5:908–918
5. Bennett J, Catovsky D (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51:189–199
6. Bloom E (1972) Further definition by cytotoxicity tests of cell surface antigens of human sarcomas in culture. Cancer Res 32:960–967
7. Carden MJ, Trojanowski JQ, Schlaepfer WW, Lee V-Y (1987) Two-stage expression of neurofilament polypeptides during rat neurogenesis with early establishment of adult phosphorylation patterns. J Neurosci 7:3489–3504
8. Cavazzana AO, Miser JC, Jefferson J, Triche TJ (1987) Experimental evidence for neural origin of Ewing's sarcoma of bone. Am J Pathol 127:507–518
9. Dehner LP (1993) Primitive neuroectodermal tumour and Ewing's sarcoma. Am J Surg Pathol 17:1–13
10. Delattre O, Zucman J, Plougastel B, Desmeze C, Melot T, Peter M, Kovar H, Joubert I, de Jong P, Rouleau G, Aurias A, Thomas G (1992) Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 359:162–165
11. Delattre O, Zucmann J, Melot T, Garau XS, Zucker J, Lenoir GM, Ambros PF, Sheer D, Turc-Carel C, Triche TJ, Aurias A, Thomas G (1994) The Ewing family of tumours – a subgroup of small-round-cell tumours defined by specific chimeric transcripts. New Engl J Med 331:294–299
12. Dockhorn-Dworniczak B, Schäfer K-L, Dantcheva R, Blasius S, Winkelmann W, Strehl S, Burdach S, Valen F, Jürgens H, Böcker W (1994) Diagnostic value of the molecular genetic detection of the t(11;22) translocation in Ewing's tumours. Virchows Arch 425:107–112
13. Enzinger FM, Weiss SW (1988) Soft tissue tumour. C.V. Mosby, St. Louis, pp 1–18, 806–815, 951–958
14. Ewing J (1921) Diffuse endothelioma of bone. Proc NY Pathol Soc 21:17–24
15. Fujii Y, Hongo T, Nakagawa Y, Nasuda K, Mizuno Y, Igarashi Y, Naito Y, Maeda M (1989) Cell culture of small round cell tumour originating in the thoracopulmonary resion. Evidence for derivation from a primitive pluripotent cell. Cancer 64:43–51
16. Gardinier MV, Macklin WB, Diniak AJ, Deininger PL (1986) Characterization of myelin proteolipid mRNAs in normal and jimpy mice. Mol Cell Biol 6:3755–3762
17. Garin-Chesa P, Rettig WJ, Thomson TM, Old LJ, Melamed MR (1988) Immunohistochemical analysis of nerve growth factor receptor expression in normal and malignant human tissues. J Histochem Cytochem 36:383–389
18. Hara S, Adachi Y, Kaneko Y, Fujimoto J, Hata J (1991) Evidence for heterogenous groups of neuronal differentiation of Ewing's sarcoma. Br J Cancer 64:1025–1030
19. Hara S, Ishii E, Fujimoto J, Hata J (1989) A monoclonal antibody specifically reactive with Ewing's sarcoma. Br J Cancer 60:875–879
20. Hasegawa T, Hirose T, Kudo E, Hizawa K, Yamawaki S, Ishii S (1991) Atypical primitive neuroectodermal tumors. Comparative light and electron microscopic and immunohistochemical studies on peripheral neuroepitheliomas and Ewing's sarcomas. Acta Pathol Jpn 41:444–454
21. Hempstead BL, Schleifer LS, Chao M (1989) Expression of functional nerve growth factor receptors after gene transfer. Science 243:373–375
22. Homma C, Kaneko Y, Sekine K, Hara S, Hata J, Sakurai M (1989) Establishment and characterization of a small round cell sarcoma cell line, SCCH-196, with t(11; 22)(q24; q12). Jpn J Cancer Res 80:861–865
23. Hu MCT, S.B. S, Davidson N (1986) The complete sequence of the mouse skeletal alpha-actin gene reveals several con-

served and inverted repeat sequences outside of the protein-coding region. Mol Cell Biol 6:15–25
24. Ikenaka K, Nakahira K, Takayama C, Wada K, Hatanaka H, Mikoshiba K (1990) Nerve growth factor rapidly induces expression of the 68-kDa neurofilament gene by posttranscriptional modification in PC12h-R cells. J Biol Chem 265: 19782–19785
25. Jaffe R, Agostini J, Santamaria M (1984) The neuroectodermal tumour of bone. Am J Surg Pathol 8:885–898
26. Jeon I-S, Davis JN, Braun BS, Sublett JE, Roussel MF, Denny CT, Shapiro DN (1995) A variant Ewing's sarcoma translocation (7; 22) fuses the EWS gene to the ETS gene ETV1. Oncogene 10:1229–1234
27. Julien JP, Ramachandran K, Grosveld FG (1985) Cloning of a cDNA encoding the smallest neurofilament protein. Biochim Biophys Acta 825:398–404
28. Kawaguchi K, Koike M (1986) Neuron-specific enolase and Leu-7 immunoreactive small round-cell neoplasms. The relationship to Ewing's sarcoma in bone and soft tissues. Am J Clin Pathol 86:79–83
29. Levi-Montalcini R (1987) The nerve growth factor. Thirty-five years later. EMBO J 6:1145–1154
30. Lewis SA, Cowan NJ (1985) Genetics, evolution, and expression of the 68,000 mol-wt neurofilament protein. Isolation of a cloned cDNA probe. J Cell Biol 100:843–850
31. Lipinski M, Braham K, Philip I, Wiels J, Philip T, Goridis C, Lenoir GM, Tursz T (1987) Neuroectoderm-associated antigens on Ewing's sarcoma cell lines. Cancer Res 47:183–187
32. Lizard-Nacol S, Lizard G, Justrabo E, Turc-Carel C (1989) Immunologic characterization of Ewing's sarcoma using mesenchymal and neural markers. Am J Pathol 135:847–855
33. Llombart-Bosch A, Lacombe MJ, Contesso G, Peydro-Olaya A (1987) Small round blue cell sarcoma of bone mimicking atypical Ewing's sarcoma with neuroectodermal features. An analysis of five cases with immunohistochemical and electron microscopic support. Cancer 60:1570–1582
34. Llombart-Bosch A, Lacombe MJ, Peydro-Olaya A, Perz-Bacete M, Contesso G (1988) Malignant peripheral neuroectodermal tumours of bone other than Askin's neoplasm: characterization of 14 new cases with immunohistochemistry and electron miroscope. Virchows Arch [A] 412:421–430
35. Marina NM, Etcubanas E, Parham DM, Bowman LC, Green A (1989) Peripheral primitive neuroectodermal tumour (peripheral neuroepitehlioma) in children. A review of the St. Jude experience and controversies in diagnosis and management. Cancer 64:1952–1960
36. Mierau G (1985) Extraskeletal Ewing's sarcoma (peripheral neuroepithelioma). Ultrastruct Pathol 9:91–98
37. Misko TP, Radeke MJ, Shooter EM (1988) Neural development and regeneration. Springer-Verlag, Berlin, pp 257-272
38. Myers MW, Lazzarini RA, Lee VM-Y, Schaepfer WW, Nelson DL (1987) The human mid-size neurofilament subunit. A repeated protein sequence and the relationship of its gene to the interfilament gene family. EMBO J 6:1617–1626
39. Nakakihara K, Ikenaka K, Wada K, Tamura T, Furuichi T, Mikoshiba K (1990) Structure of the 68-kDa neurofilament gene and regulation of its expression. J Biol Chem 265:19786–19791
40. Noguera R, Navarro S, Peydro OA, Llombart BA (1994) Patterns of differentiation in extraosseous Ewing's sarcoma cells. An in vitro study. Cancer 73:616–24
41. Noguera R, Triche TJ, Navarro S, Tsokos M, Llombart BA (1992) Dynamic model of differentiation in Ewing's sarcoma cells. Comparative analysis of morphologic, immunocytochemical, and oncogene expression parameters. Lab Invest 66: 143–151
42. Okuda T, Kasai K, Kameya T, Saito S, Takase N (1988) Monoclonal antibody directed against neuroendocrine properties of both normal and malignant cells. Hybridoma 7:569–581
43. Plougastel B, Zucman J, Peter M, Thomas G, Delattre O (1993) Genomic structure of the EWS gene and its relationship to EWSR1, a site of tumour-associated chromosome translocation. Genomics 18:609–615
44. Rao VN, Papas TS, Reddy SP (1987) ERG, A human ets-related gene on chromosome 21: alternative splicing, polyadenylation, and translation. Science 237:635–639
45. Schlaepfer WW, Bruce J (1990) Simultaneous up-regulation of neurofilament proteins during the postnatal development of the rat nervous system. J Neurosci Res 25:39–49
46. Schmidt D, Herrmann C, Jurgens H, Harms D (1991) Malignant peripheral neuroectodermal tumour and its necessary distinction from Ewing's sarcoma. A report from the Kiel Pediatric Tumor Registry. Cancer 68:2251–2259
47. Shaw G, Weber K (1982) Differential expression of neurofilament triplet proteins in brain developmen.. Nature 298:277–279
48. Shimada H, Newton WA, Soule EH, Qualman SJ, Aoyama C, Mauer HM (1988) Pathologic features of extraosseous Ewing's sarcoma: a report from the Intergroup Rhabdomyosarcoma Study. Human Pathol 19:442–453
49. Sorensen PHB, Lessnick SL, Lopez-Terrada D, Liu XF, Triche TJ, Denny CT (1994) A second Ewing's sarcoma transocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor, ERG. Nature Genetics 6:146–151
50. Steinert PM, Jones JCR, Goldman RD (1984) Intermediate filament. J Cell Biol 99:22–27
51. Trojanovski JQ, Walkenstein N, Lee VM-Y (1986) Expression of neurofilament subunits in neurons of the central and peripheral nervous system. An immunohistochemical study with monoclonal antibodies. J Neurosci 6:650–660
52. Turc-Carel C, Philip I, Berger MP, Lemoir GM (1983) Chromosomal translocation Ewing's sarcoma. New Engl J Med 309:497–498
53. Umezawa A, Hata J (1992) Expression of gap-junctional protein (connexin 43 or alpha 1 gap junction) is down-regulated at the transcriptional level during adipocyte differentiation of H-1/A marrow stromal cells. Cell Struct Funct 17:177–184
54. Umezawa A, Tachibana K, Harigaya K, Kusakari S, Kato S, Watanabe Y, Takano T (1991) Colony-stimulating factor 1 expression is down-regulated during the adipocyte differentiation of H-1/A marrow stromal cells and induced by cachectin/tumor necrosis factor. Mol Cell Biol 11:920–927
55. Urano F, Umezawa A, Hong W, Kikuchi H, Hata J-i (1996) A novel chimera gene between EWS and E1A-F, encoding the adenovirus E1A enhancer-binding protein, in extraosseous Ewing's sarcoma. Biochem Biophy Res Comm 219:608–612
56. Ushigome S, Shimoda T, Nikaido T, Nakamori K, Miyazawa Y, Shishikura A, Takakuwa T, Ubayama Y, Spjut HJ (1992) Primitive neuroectodermal tumorus of bone and soft tissue. With reference to histologic differentiation in primary or metastatic foci. Acta Pathol Jpn 42:483–493
57. Whang-Peng J, Triche TJ, Knusten T, Miser J, Douglas EC, Israel MA (1984) Chromosomal translocation in peripheral neuroepithelioma. New Engl J Med 311:584–585