

Cytogenetic investigations of solid tumours of children

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SUMMARY The association of non-random chromosome abnormalities with solid tumours of childhood may improve accuracy of diagnosis and prognosis and lead to a better understanding of their biology. In a pilot study in the Northern region of England fresh tumour biopsy specimens were obtained from 39 of 72 consecutive solid tumours in children who presented over a period of 21 months. Cytogenetic analysis was possible in 33 and clonal chromosomal abnormalities were detected in nine. In addition, seven of 10 tumours investigated after treatment were abnormal. Ten of these 16 abnormal karyotypes have not previously been described. This pilot study has shown that a concerted investigation of tumour cytogenetics is possible. A multicentre study is essential if our knowledge of basic tumour cytogenetics is to progress.

Despite our ability to achieve prolonged remissions in most children with cancer, malignant disease remains second only to birth defects as the most common non-accidental cause of death in children over the age of 1 year in the United Kingdom. Accurate diagnosis is essential in order that appropriate treatment is used, but even within any one tumour category there remain variations in behaviour and response to treatment.

In the leukaemias some clonal cytogenetic abnormalities have already proved to be of diagnostic and prognostic value. For example, the translocations t(9;22), t(8;21), t(15;17), and t(8;14) help towards the diagnosis of chronic myeloid, acute myeloblastic, acute promyelocytic, and Burkitt's leukaemia respectively,¹ and clonal structural abnormalities are associated with a poor outcome in acute lymphoblastic leukaemia of childhood.² Studies of the oncogene c-abl in chronic myeloid leukaemia³ and the relationship between c-myc and immunoglobulin gene loci in Burkitt's lymphoma⁴ have led to a rapid increase in our knowledge of the molecular biology of haemopoietic malignancy.

Although clonal cytogenetic abnormalities have been reported in some solid tumours of children, much of the published information has appeared as single case reports. Cytogenetic investigations of solid tumours lag far behind those of leukaemias due to a combination of reasons including technical problems and the relative difficulty in obtaining viable tumour cells. One of the early steps in

understanding the biology of malignancy, however, is to obtain a detailed knowledge of non-random chromosomal abnormalities of tumour cells. If the analogy with leukaemia is appropriate, then cytogenetic studies of solid tumours could provide diagnostic and prognostic information and the basis for a detailed study of the molecular events associated with their genesis.

In order that this baseline knowledge can be obtained a vigorous and comprehensive effort to study the chromosomes of all childhood solid tumours is required. We have embarked on a pilot study to obtain fresh material from most childhood solid tumours in the Northern region and carry out cytogenetic studies on these specimens. This report describes our initial results.

Methods

COLLECTION OF FRESH TUMOUR TISSUE

This was carried out in two phases. In January 1986 paediatric surgeons and pathologists within Newcastle were asked to inform us whenever a suspected tumour in a child was to be biopsied. We requested that the biopsy specimen be as generous as the surgeon could provide and be transferred fresh or in tissue culture medium (such as RPMI or Dulbecco's minimum essential medium) to a specified pathologist. A portion of the macroscopically viable tissue was transferred to the cytogenetics unit.

The second phase commenced in September 1986

when all paediatricians within the Northern region and many of the pathologists were invited by letter to participate.

The success in obtaining fresh tumour material was assessed by comparing the number of samples received with the total number of tumours notified to the Northern region Children's Malignant Disease Registry.⁵

CYTOGENETIC METHODS

Tumour was dispersed by gentle pipetting to provide a cell suspension for direct and short term cultures (one to 48 hours). Long term explant cultures were established in plastic Falcon flasks using various culture media (Ham's F-10, Chang's, and Leibovitz L-15, all supplemented with 20% fetal bovine serum) and incubated at 37°C. Colcemid exposure before harvesting varied between 0.2 µg/ml for 15 minutes for direct cultures and 0.01 µg/ml for four hours for long term cultures. Slide preparations were made by standard techniques and G banded with trypsin and Leishman's stain.

Results

COLLECTION OF FRESH TUMOUR TISSUE

From January 1986 to September 1987 inclusive, 72 new solid tumours occurring in children under the age of 15 years were notified to the Northern region Children's Malignant Disease Registry. Fresh tissue, either primary or metastatic, was obtained from 39 of these 72 solid tumours (table 1). During the same period fresh tumour was obtained from eight of 10 relapsed tumours from which tissue was available and from two of four children who had a biopsy of the tumour remaining after initial chemotherapy.

CYTOGENETIC ANALYSIS

Thirty seven specimens obtained at diagnosis were assessed; analysable metaphases were obtained from 33. One patient with a neuroblastoma had a constitutional abnormality [46,XX t(8;11)

(q22.1;q21)]. Clonal cytogenetic abnormalities were found in nine comprising two neuroblastomas, two brain tumours, and one each of retinoblastoma, Ewing's sarcoma, Wilms' tumour, osteogenic sarcoma, and lymphoma (table 2). The remaining 23 were karyotypically normal apart from several single cell abnormalities.

Analysable metaphases were obtained from six of eight relapsed tumours studied, five of which were abnormal and both specimens obtained from second look procedures had abnormal karyotypes. Overall 16 out of 47 tumours studied had abnormal karyotypes (table 2).

Discussion

The aim of this pilot study was twofold. Firstly it was to assess the feasibility of collecting fresh viable tissue from most childhood solid tumours in the Northern region and secondly, to carry out cytogenetic analysis of the specimens. This study has shown that both aspects are feasible with the cooperation of regional paediatricians, surgeons, and pathologists. Fresh material has been obtained from most tumours, and the use of a limited number of standard techniques by a single cytogenetics department has resulted in good quality analysable metaphase preparations in most cases.

No single culture technique has so far proved superior. Further experiments may show optimal culture techniques for each tumour type. Many of the cultures showed karyotypically normal cells. These may well represent non-malignant cells, such as fibroblasts, within the tumour. We believe, however, the clonal abnormalities obtained from tumour tissue at diagnosis did originate from tumour cells. Until larger numbers have been studied we cannot be certain that some clones do not represent a secondary abnormality, occurring in a genetically unstable tumour cell, brought about and selected by the culture conditions. Particular caution is necessary in interpreting clonal abnormalities in tissues that have been subjected to chemotherapy or radiotherapy, or both.

None the less, the cytogenetic abnormalities found in cells from a heavily pretreated neuroblastoma primary tumour,⁶ bone marrow metastases from another, and spinal fluid seedlings from a relapsed medulloblastoma are characteristic of those in untreated tumours.⁷ Also two of the clones (B and C) from a relapsed ependymoma (data not in table) resemble the abnormalities described in the only other published G banded case of this disease.⁸ In all other cases, apart from the two remaining disseminated neuroblastomas, the abnormalities we have found have not previously been described. Of

Table 1 *Details of solid tumours presenting in the Northern region, January 1986–September 1987*

	<i>No of children</i>	<i>No in which fresh tumour tissue obtained</i>
Biopsy of primary tumour	50	24
Biopsy of metastatic disease	14	14
Biopsy of primary and metastatic tumour	1	1
No biopsy	7	—
Total	72	39

Table 2 Abnormal karyotypes from childhood solid tumours

	Patient status at time of study	Karyotype
Primary tumour		
<i>Long term cultures</i>		
Retinoblastoma	ND	46,XX/46,XX t(6;11)(p21.3;p17.5)/92,XXXX
Osteogenic sarcoma	ND	46,XY t(2;3)(p13;q24)
Ependymoma	ND	45,XY,-16,t(6;16)/46,XY (~50% cells endoreduplicated)
Wilms' tumour	ND	51,XX,+8,+12,+12,+13,+17, 51,XX,+8,+12,+12,+13,+17,t(1;16)(p3;p22)
Wilms' tumour	PC	46,XY/46,XY,t(19;21)/46,XY,t(10;10)
Neuroblastoma	PC	46,XY/46,XY,1p+/46,XY,1p+,t(4;10),17p+
Primitive neuroectodermal tumour	R	46,XY,t(3;10),inv(7)(q32;p22)/46,XY,+ markers including ?del(2)(q31), ?t(1;18)(q1?;q21), inv(5)(q22;q23), ?del(14)(q22)
Ependymoma	R	6 different clones each with multiple markers*
Rhabdomyosarcoma	R	82,XX,+2,2,2,2,4,5,5,6,6,7,8,10,12,13, 14,15,15,18,18,19,20,21,22,22,+X,+M ₁ , M ₂ ,M ₃ ,M ₅ ,M ₅ ,M ₆ ,M ₇
Metastatic tumour (site)		
<i>Direct cultures</i>		
T cell lymphoma (pleural fluid)	ND	45,XY+ multiple markers/87,XY+ multiple markers
Ewing's sarcoma (bone marrow)	ND	46,XX/45,XX,-7,-10,-21,-22,+M ₁ ?del 7,M ₂ +M ₃
Neuroblastoma (bone marrow)	ND	46,XX,-7,-22,+M ₁ +M ₂
Neuroblastoma (bone marrow)	ND	46,XX/polyploids with double minutes
Medulloblastoma (bone marrow)	ND	46,XX/polyploids with double minutes
Medulloblastoma (cerebrospinal fluid)	R	50,XX,+5,+6,+8,+13,-11,-17,-22,+11q, +22q,+i(17q)/49,XX,+5,+6,+8,-11,-17, -22,+11q+,+11q+,-22p+,-i(17q)
Neuroblastoma (bone marrow)	R	46,XY/double minutes

ND=newly diagnosed; R=relapse; PC=postchemotherapy.

*Details of these clones are available on request.

particular interest is the primitive neuroectodermal tumour (PNET), full details of which are being published elsewhere.⁹ Potluri *et al* have suggested that PNETs may be subdivided by their cytogenetic features into peripheral neuroepitheliomas with a t(11;22) and other PNETs showing other karyotypic abnormalities.¹⁰ The tumour described here falls into the latter group.

This series of karyotypes highlights the current state of knowledge of cytogenetic abnormalities of solid tumours of childhood; each case constitutes a significant contribution to our knowledge. Although karyotypic abnormalities are being reported increasingly at international scientific meetings, most are published only as abstracts. Some patterns have begun to emerge. In retinoblastoma, isochromosome 6p appears to be a unique abnormality,¹¹ and the one case of retinoblastoma in the present series also had an abnormality of chromosome 6. Alveolar rhabdomyosarcomas are associated with t(2;13),¹²

and evidence suggests that 3p 14 → 21 may be a non-random site for change in this disease.¹³ In addition to PNETs t(11;22) is associated with Ewing's sarcoma in some but not all cases.¹⁴ This association raises the possibility that Ewing's sarcoma and some forms of PNETs have a common origin. In translocations involving chromosome 22 the breakpoint arises in the region of the oncogene *c-cis*.¹⁵ The Ewing's tumour described in this report did not have this translocation but did have an abnormal chromosome 22.

The association of aniridia and Wilms' tumour has been linked with constitutional deletion of 11p13. Abnormalities of this chromosomal region have been detected in some Wilms' tumours from children with a normal constitutional karyotype.¹⁶ Additional abnormalities of chromosomes 1 and 16 may occur in advanced tumours.^{16 17} Recently the combination of hyperdiploidy, complex chromosomal translocations, and an anaplastic histological

appearance of Wilms' tumour have been associated with a particularly poor prognosis.¹⁸

After leukaemias, brain tumours remain the single largest group of malignancies in children. Until recently investigation of these tumours has been hampered by problems of nomenclature and classification. Others have already begun to approach the problem of improving diagnosis and prognosis and understanding their biology by undertaking cytogenetic investigations.¹⁹

The differential diagnosis of small round cell tumours of childhood includes rhabdomyosarcoma, lymphoma, Ewing's sarcoma, and neuroectodermal tumours. Despite the increasing use of specialised investigations, including monoclonal antibodies and electron microscopy, the identity of some tumours remains obscure.²⁰ As in haemopoietic malignancy, cytogenetic investigation of solid tumours may emerge as an important diagnostic tool. At present prognostic factors in most children with solid tumours are limited to clinical and histopathological staging and in some cases histological subtyping.^{21 22} Cytogenetic abnormalities may provide additional prognostic information, but until many hundreds of tumours have been karyotyped we cannot begin to approach the level of diagnostic and prognostic value that now exists for leukaemias. Despite this relative lack of knowledge, the ability to detect cytogenetic abnormalities in metastatic sites and treated primary tumours may improve the accuracy of staging procedures and assessment of response to treatment.⁶ This comparatively simple aspect of the value of cytogenetic investigations has so far not been exploited.

In conclusion, it is recommended that biopsy material from tumours in children should never automatically be put into formalin. The Northern region pilot study has shown that fresh biopsy material can be obtained from most childhood solid tumours and cytogenetic investigation can be successful in a wide variety of cancers. Cooperation between groups of workers in the field of paediatric oncology could lead to multicentre studies of fundamental aspects of the biology of childhood cancer, one of which is the investigation of cytogenetic abnormalities.

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