Recent advances in the diagnosis, prognosis and classification of childhood solid tumours

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The diagnosis of paediatric tumours including the small round cell tumours (neuroblastoma, rhabdomyosarcoma and the Ewing family of tumours), brain tumours, germ cell tumours and anaplastic large cell lymphoma can pose particular diagnostic dilemmas, especially in cases with undifferentiated morphology. Substantial improvements have been made in the treatment and long term survival of paediatric patients with these tumours, however, these are based on disease and even stage specific treatments. Accurate diagnosis and prognosis can now be aided by identifying specific genotypic and phenotypic criteria using cytogenetics, interphase fluorescence in situ hybridisation, reverse transcription PCR and novel immunophenotypic markers. Some of these analyses should form an integral part of the management of patients with paediatric solid tumours.

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The diagnosis of some paediatric tumours can be difficult using the standard approaches of light microscopy, electron microscopy and immunohistochemistry, particularly in cases with an undifferentiated morphology. Accurate diagnosis is increasingly important with the development of disease-specific therapeutic strategies. Consistent, specific chromosome rearrangements are associated with particular tumour types and are a visible hallmark of the underlying genetic changes. Chromosome rearrangements include deletions, translocations and gain or amplification of chromosomal segments. At the molecular level, these rearrangements are associated with loss of tumour suppressor genes, the formation of novel fusion gene products and the overexpression of genes. Increasingly, identification of these characteristic genetic changes at the cytogenetic or molecular level are recognised as invaluable diagnostic indicators. In addition, rearrangements may also be associated with different prognostic groups and be of clinical significance. Chromosome rearrangements have been used for many years in the diagnosis and management of haematological malignancies but a similar approach for solid tumours has been hampered by difficulties in the preparation and analysis of chromosome preparations from solid tumours. Following the relatively recent molecular characterisation of various tumour specific
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rearrangements, strategies which negate the need for chromosome preparation can now be used to identify particular molecular abnormalities in non-dividing cells. Unique phenotypic markers, which are associated with tumour specific molecular changes, have also been identified which have diagnostic and prognostic value. This article describes these recent advances which are relevant to the diagnosis, prognosis and also the classification of paediatric solid tumours.

Methodology

The main approaches used to identify genetic rearrangements and phenotypic markers used in the management of patients with solid tumours are described below.

Cytogenetics

Fresh material is required for cytogenetic analysis and usually involves disaggregation of tumour cells, either mechanically or enzymatically. The cells can be either harvested directly or cultured for various lengths of time in specialised media although the latter can selectively encourage the growth of particular cell types, including contaminating normal cells. Colchicine, or a related agent, is added to the cells prior to harvest in order to stop mitotic spindle formation and increase the number of cells at, or just prior to, the metaphase stage of mitosis. The cells are then swollen in hypotonic solution and fixed either in situ or in suspension. Various banding techniques allow individual chromosomes and rearrangements to be recognised (Fig. 1A).

Fluorescence in situ hybridisation (FISH)

In situ hybridisation involves the chemical modification of nucleic acid probes and their hybridisation to homologous single stranded nucleic acid target, usually on a microscope slide. The nature and position of the hybridized probe can then be detected relative to chromosome or cellular morphology or relative to the signals from other probes. For example, if two probes normally lie adjacent to one another but are split by a reciprocal translocation, an interphase nucleus will show two signals adjacent, corresponding to the normal chromosome, and one pair of split signals indicative of the rearrangement at that locus (Fig. 2A,B). Many
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A. t(2;13)(q35;ql4)

2 der(2) 13 der(13)

- 158bp

Fig. 1 Cytogenetic and molecular analysis of alveolar rhabdomyosarcoma sample. (A) Partial karyotype showing the typical t(2;13)(q35;q14) rearrangement. (B) Reverse transcription PCR analysis of the same case. No product is present using primers for the t(1;13)(p36;q14) rearrangement in lane 1, a 158 bp product is found using primers for the t(2;13)(q35;q14) rearrangement in lane 2 and no product in the control reaction, lane 3 which had no DNA and primers for the t(2;13).

variations on this theme, using different probe types, are possible and can be used to identify specific rearrangements and copy number changes.

**Reverse transcription PCR (RT-PCR)**

Reverse transcription PCR can be used to detect the aberrant fusion products that result from specific translocations. The approach is dependant on isolating intact RNA which is copied by the enzyme reverse transcriptase into complementary DNA (cDNA). An appropriate primer from each of the two genes disrupted by a translocation can be used to amplify the fusion gene using the polymerase chain reaction. The product can be visualised as a discreet band on an agarose gel and only material containing the tumour-specific fusion gene should produce a product (Fig. 1B). The technique is very sensitive and is able to detect one cell with a rearrangement out of approximately $10^5$–$10^6$ normal cells.

**Loss of heterozygosity (LOH)**

In the two hit hypothesis for tumour development, loss of function of both copies of a tumour suppressor gene was postulated and has now been demonstrated in many tumours. The first hit usually involves a mutation in one copy of the gene and the second hit results in loss of a large region of DNA including the second copy of the gene. Using region specific polymorphic markers, this loss of DNA can be detected in tumour samples as homozygosity for a locus compared to the
constitutential heterozygosity of the patient. This can be identified in blot-hybridisation experiments or using the polymerase chain reaction.

**Immunohistochemistry (IHC)**

This is a histological technique to localise gene products in tissue sections, cytospin or touch preparations. Its main advantage lies in the precise identification of the cell type (tumour versus stromal cell or benign versus malignant). Recent advances in IHC include the availability of new antibodies (for diagnostic or prognostic purposes) and newer antigen-retrieval methods such as using microwaves or hydrated autoclaving using pressure cookers to unmask epitopes in routinely processed tissue sections.

**Small round cell tumours (SRCT)**

The small round cell tumours include neuroblastoma, rhabdomyosarcoma and the Ewing family of tumours and represent about 15% of all childhood cancers. They can be difficult to distinguish from one another by standard methods but fortunately possess characteristic molecular rearrangements which can be used as diagnostic and prognostic indicators (reviewed in).

**Neuroblastoma**

Neuroblastoma is the most common extracranial solid malignant tumour of childhood and originates from the sympathetic neurons. Prognostic categories have been correlated with distinct clinical, biological and genetic factors which can influence the therapeutic regimens adopted.

1p36 loss, ploidy and MYCN amplification: The most common cytogenetic abnormality in neuroblastoma, found in 70–80% of cases, is deletion or rearrangement of material from 1p. Approximately 25–30% of all neuroblastomas show cytogenetic evidence of gene amplification, most usually in the form of extrachromosomal double minutes (dms) but also as homogenously staining regions (hsrs) which are attributed to additional copies of the MYCN gene. This is associated with high MYCN expression at the RNA and protein level. Both MYCN amplification and loss of 1p material correlate with poor outcome and have been identified by the International Neuroblastoma Staging System.
Table 1 Summary of the main genetic and phenotypic markers used to aid the diagnosis and predict the behaviour of paediatric tumours

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Sub-type</th>
<th>Cytogenetic rearrangement</th>
<th>Molecular rearrangement</th>
<th>IHC</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma</td>
<td></td>
<td>3n</td>
<td>MYCN amplification</td>
<td>High TRKA</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Del(1) 2n, 4n</td>
<td></td>
<td>Low TRKA</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Del(11) dms and hrs 2n,4n</td>
<td></td>
<td>Low TRKA</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Embryonal</td>
<td>t(1;13)(q35;q14)</td>
<td>PAX3/FKHR</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Alveolar and solid alveolar</td>
<td>t(11;22)(q12;q22)</td>
<td>FLI1/EWS</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>Multiploid</td>
<td>t(21;22)(q22;q12)</td>
<td>ERG/EWS</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>Ewing family of tumours</td>
<td></td>
<td>2n</td>
<td>MYF5 over expression</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>pPNET</td>
<td></td>
<td>t(11;22)(p13;q12)</td>
<td>FLI1/EWS</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>Biphenotypic sarcomas</td>
<td></td>
<td>i(17p)</td>
<td>TP53</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>MEM</td>
<td></td>
<td>t(11;22)(q24;q14)</td>
<td>PAX7/FKHR</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>DSRCT</td>
<td></td>
<td>t(1;13)(p36;q14)</td>
<td>LOH 11p</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>PNET</td>
<td>i(17p)</td>
<td>t(l;13)(p36;q14)</td>
<td>CD44</td>
<td></td>
<td>Good</td>
</tr>
<tr>
<td>GCT</td>
<td>i(12p)/del(1)</td>
<td></td>
<td>Pgp</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>ALCCL</td>
<td>t(2;5)(p23;q35)</td>
<td></td>
<td>TP53 intact</td>
<td></td>
<td>Better than cases with mutations</td>
</tr>
</tbody>
</table>

aPNET, peripheral primitive neuroectodermal tumour. MEM, malignant ectomesenchymoma. DSRCT, desmoplastic small round cell tumour. PNET, primitive neuroectodermal tumours. GCT, germ cell tumour. ALCCL, anaplastic large cell lymphoma. dms, double minutes. hrs, homogenously staining regions.

and Response Criteria Committee as important factors to take into account. Favourable prognosis is associated with a young age and early stage (1, 2a or 4s), a near triploid karyotype and no 1p rearrangements or MYCN amplification. Poor prognosis in neuroblastoma is associated with older age, advanced stage (2b, 3 or 4), near diploidy or tetraploidy and 1p loss and MYCN amplification (Table 1).

Current evidence indicates a consensus deletion at the 1p36.2–3 region and that some cases also have deletion of a more proximal locus. It is proposed that two tumour suppressor genes are located in the 1p32–p36 region although none have so far been implicated. In cases showing a larger deletion, 60% had MYCN amplification, were near diploid or tetraploidy and were associated with a poor prognosis.

Cytogenetic analysis is generally successful in less than 25% of cases and the more practical alternatives of interphase FISH to tumour touch imprints (Fig. 2E), bone marrow smears and paraffin sections to identify the specific genetic changes associated with neuroblastoma have been demonstrated. Allele loss can also be used to investigate loss of 1p material and the copy number of MYCN also estimated from
hybridisation of probes to DNA on membranes. Amplification of MYCN is generally associated with increased expression which can be estimated by a differential polymerase chain reaction and reactivity to an antibody specific to the MYCN protein (reviewed in3).

**TRKA expression:** Neurotrophins comprise a family of growth factors which play an important role in the development of the nervous system7,8. The biological functions of neurotrophins are mediated by high affinity binding to tyrosine kinase transmembrane receptors encoded by the TRKA protooncogene. TRKA receptors show specific differences in distribution and ligand specificity. Nerve growth factor (NGF), the best characterised neurotrophic factor, is essential for the development, differentiation and survival of the sympathetic neurons. The biologic responsiveness to NGF depends on interactions with TRKA (p140frt) which is an important component of the high-affinity NGF receptor7,8.

A high level of TRKA expression, assessed by Northern blot analysis, was seen in 82% of neuroblastomas and correlated strongly with favourable prognostic features (early tumour stage, younger age, normal MYCN copy number and low level of MYCN expression7,8). The expression of TRKA also correlated strongly with survival and in a univariate analysis it has emerged, together with MYCN copy number, as the most powerful predictor of outcome8.

Recently, IHC techniques using a monoclonal antibody9 or commercially available polyclonal antibodies10 have been utilised for the detection of p140frt in frozen9 or formalin-fixed neuroblastoma tumour samples10. In one study, all the 29 tumours showed variable immunoreactivity of tumour cells with p140frt and the intensity of staining increased with maturation of the neuroblasts10. A semiquantitative analysis of p140frt expression correlated with a statistically significant increase in survival and with favourable tumour stages10.

**CD44 expression:** CD44 is a transmembrane glycoprotein which may be present as a standard molecule (CD44s) or as various isoforms (CD44v)11. It is multifunctional cell-adhesion molecule and plays a key role in lymphocyte homing, cell–cell interactions, tumour progression and metastasis. IHC employing antibodies directed against both the molecules have detected only CD44s immunoreactivity in a variable number of neuroblastoma tumour samples11.

In a study12 comprising of 52 cases, CD44 immunoreactivity was detected in 71% of the neuroblastoma samples. All the 22 tumours in good prognosis stages (1, 2 or 4s) but only 50% (15/30) of advanced neuroblastomas (stages 3 and 4) expressed CD44. The cumulative progression-free survival was significantly higher in CD44 positive patients and within the high-risk group, the progression-free survival
Correlated significantly with survival. CD44 expression emerged as an independent prognostic variable in univariate and multivariate analyses of 5 prognostic factors including tumour stage, age, histology and MYCN amplification. Another group detected CD44 immunoreactivity in 86% of the 377 neuroblastoma samples. However, it was a marker of favourable outcome only in a univariate but not in a multivariate analysis which included other molecular variables (MYCN amplification, cytogenetic chromosome 1p deletion, loss of 1p heterozygosity and DNA ploidy).

CD44 immunoreactivity also correlated significantly with grade of tumour-cell differentiation and one-third of the stroma-poor undifferentiated tumours showed lack of expression. The event-free survival of patients in this group was significantly shorter than that of patients with CD44 positive tumours. These studies suggest that CD44s expression may be a new histological marker in neuroblastoma having a prognostic impact in tumours of undifferentiated histology.

**Multidrug resistance (MDR) expression:** Overexpression of the multidrug resistance gene, *MDR1* and its product P-glycoprotein (Pgp or p170) in clinical neuroblastoma samples may be predictive of poor prognosis. Using a sensitive IHC technique, Pgp was not only detected in 63% of the patients with neuroblastoma but its expression before treatment suggested failure of chemotherapy. Other workers using *in situ* hybridisation and IHC detected *MDR1* mRNA and Pgp in all the neuroblastoma cases and there was no correlation with age, stage or survival. A non-Pgp mediated form of MDR, p110, was detected immunocytochemically in ganglion cells but there was no correlation with survival.

**BCL2 expression:** BCL2 gene encodes an oncoprotein (BCL-2) which blocks apoptosis or programmed cell death and renders cells markedly resistant to killing by a wide variety of cytotoxic drugs. IHC studies have detected BCL-2 in neuroblastoma tumour samples. BCL-2 immunolocalisation strongly correlated with unfavourable histology and MYCN amplification in one study but no correlation was found between BCL-2 immunostaining and other prognostic markers such as Shimada histology, tumour stage or patient age in other studies. The intensity of staining increased with the degree of differentiation of neurons and was reciprocal to that of MYCN protein.

**NB84 expression:** This monoclonal antibody recognises an uncharacterised molecule of 57kDa and was produced using human neuroblastoma tissue as an antigen. It works in formalin-fixed, paraffin-embedded sections and has recently become commercially available.
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is highly sensitive as it identifies 95% of the neuroblastoma samples. However, it lacks specificity as it also marks a high proportion of frozen and routinely-processed Ewing’s sarcoma samples (personal observation). Therefore, it should be included in a panel of markers used for the immunocytochemical identification of SRCTs.

**Rhabdomyosarcoma (RMS)**

Rhabdomyosarcoma is the most common soft tissue sarcoma in children and is a heterogenous group of neoplasms thought to be of skeletal muscle origin. The conventional classification scheme of RMS consists of botryoid, embryonal, alveolar and pleomorphic subgroups. Recently a universal classification scheme has been devised with a view to improving the prognostic significance of histology\(^{17}\). The aim was to define the criteria which would be reproducible among pathologists as well as be predictive of prognosis. The following modifications were made: (i) inclusion of the solid alveolar subtype and recognition that even a focal alveolar pattern would qualify a RMS to be classified as alveolar; (ii) identification of a new subtype of embryonal RMS with favourable prognosis and predilection to paratesticular sites called spindle-cell embryonal RMS; and (iii) exclusion of pleomorphic subtype of RMS because of its rare occurrence in children.

**MyoD1 expression:** RMS have been shown to express, to different extents, genes involved in muscle cell development and differentiation\(^{18}\). MyoD1 expression has been most consistently found and immunostaining using the monoclonal antibody against MyoD1 used in routinely processed sections now seems feasible using the microwave technique for the unmasking of antigens\(^1\). MyoD1 shows promise as a specific and sensitive marker of RMS allowing its distinction from other small round cell tumours\(^{19}\).

**Genetic changes associated with alveolar histology:** The alveolar type of rhabdomyosarcoma is associated with poor prognosis and is characterised by a t(2;13)(q35;q14) translocation. This results in the fusion of the 5' region of the PAX3 gene (located at 2q35) to the 3' sequences of the FKHR gene and the formation of a fusion gene which is implicated in the pathogenesis of the tumour. A variant t(1;13)(p36;q14) has been described and characterised as involving the PAX7 gene (located at 1p36) and the FKHR gene (reviewed in\(^3\)). RT-PCR for these rearrangements has been described, including identification of the PAX3/FKHR fusion gene in the solid alveolar subtype\(^{20}\). This approach is also
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Fig. 2 Interphase fluorescence in situ hybridisation as an approach to identify diagnostically and prognostically significant chromosome aberrations in tumour samples. (A) Diagram to show disruption of the marker-flanking the FKHR gene on chromosome 13 and translocation of the telomeric marker to chromosome 2 in alveolar rhabdomyosarcoma. (B) In interphase nuclei, the red/green doublet corresponds to the normal chromosome homologue and the split signal is indicative of the translocation. (C) An interphase nucleus from an alveolar rhabdomyosarcoma showing multiple red/green doublets corresponding to amplification of the FKHR/PAX7 fusion gene. (D) Nuclei from rhabdomyosarcoma hybridized to chromosome 2 (green) and 3 (red) centromere specific probes indicating multiploidy. (E) MYCN probe (red) and a control single copy probe (green) hybridized to a nucleus from a neuroblastoma indicating amplification of the MYCN gene. (F) Diagram to show the position of a single copy 12p marker (green) and a centromere specific probe (red) in a normal chromosome 12 and an i(12p) chromosome. The latter is associated with adolescent germ cell tumours (GCT). The size of the signal from the centromere probe is different in the i(12p) and this can be seen in interphase cells. In addition, the signal from the centromere probe is flanked by signal from the 12p marker in the i(12p) chromosome. In the example in (G) the nucleus from a GCT shows 3 copies of a normal 12 chromosome and 3 copies of an i(12p) chromosome. Images were captured using a cooled CCD camera (Photometrics, AZ, USA) and SmartCapture software from Vysis (UK) Ltd.
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appropriate for detecting bone marrow infiltration. Interphase FISH analysis using markers flanking the \textit{FKHR} and \textit{PAX3} genes has also been validated as a diagnostic aid\textsuperscript{20,21} (Fig. 2A,B). Recent evidence indicates that a high proportion of cases which have the \textit{PAX7}/\textit{FKHR} fusion gene also amplify this gene\textsuperscript{22,23}. Interphase analysis of such cases produces multiple signals from 5' \textit{PAX7} and 3' \textit{FKHR} probes (Fig. 2C). It has been suggested that the variant \textit{PAX7}/\textit{FKHR} fusion gene may be associated with later presentation and development in the extremities\textsuperscript{24}. The \textit{MYCN} gene is sometimes amplified in rhabdomyosarcoma and seems to be exclusively associated with the alveolar subtype\textsuperscript{25} (J Shipley unpublished). Unlike amplification of \textit{MYCN} in neuroblastoma, this does not appear to carry any prognostic significance, possibly because the prognosis is so poor anyway\textsuperscript{25}.

\textit{Loss of heterozygosity (LOH) at 11p}: LOH at 11p15.5 is a consistent finding in the embryonal form RMS in 13 out of 14 samples and has been suggested to be specific to this form of RMS. Evidence suggests a role for genomic imprinting in this region (i.e. the expression of a gene being determined by its parental origin) resulting in the functional equivalent of LOH (reviewed in\textsuperscript{3}).

\textit{Ploidy}: The significance of ploidy in rhabdomyosarcoma remains unclear. It is commonly assessed by flow cytometric analysis but cytogenetic and FISH analysis are also possible (Fig. 2D). Alveolar histology is generally associated with near tetraploid or diploid chromosome numbers and the embryonal form with hyperdiploidy. In a study of mixed types of rhabdomyosarcoma, an aneuploid pattern indicated favourable prognosis, tetraploidy was intermediate and patients with a diploid or multiploid pattern did poorly\textsuperscript{26}. In a group of non-metastatic embryonal rhabdomyosarcoma the hyperdiploid group was associated with a significantly better survival than the diploid tumours\textsuperscript{27}. This suggests that sub-groups of rhabdomyosarcoma identified on the basis of ploidy could benefit from refinements in risk-directed therapy.

\textit{Ewing family of tumours}

Ewing's sarcoma is the second most common malignant bone tumour in children and also occurs in extra osseous sites. It shows similarities to a diverse group of tumours referred to as peripheral primitive neuroectodermal tumours (pPNET). Ewing's sarcoma and pPNET tend to exhibit features of neuroectodermal differentiation and are characterised by specific cytogenetic rearrangements. These features aid in distinguishing
them from other small round cell tumours and strengthen the concept that they are part of the disease spectrum forming the Ewing family of tumours. The prognostic relevance of grouping Ewing’s sarcoma and pPNET together remains unclear. Poorer prognosis has been associated with increasing neuroectodermal differentiation although recent trials with stage and site matched cases of soft tissue Ewing’s sarcoma and pPNET in multi-agent chemotherapy regimens suggest that the outcomes are similar.

**EWS gene disruption:** The Ewing family of tumours are characterised by a t(11;22)(q24;q12) translocation which results in the fusion of the EWS gene, a putative RNA binding gene at 22q12, and the FLI1 gene, a member of the ETS family of transcription factors at 11q24. In a variant rearrangement, occurring in approximately 10% of cases, the EWS gene fuses to the ERG gene on chromosome 21. Fusion of EWS gene to ETV at 7p22 and E1AF at 17q12 has also been documented (reviewed in). There do not appear to be any clinical differences associated with the variant rearrangements.

In addition to these rearrangements being identified cytogenetically, they can be detected using both interphase FISH and RT-PCR analysis as an effective diagnostic aid.

**MIC2 expression:** Various antibodies to CD99 which recognise the MIC2 gene product have been employed in distinguishing this group from other SRCTs. These have the advantage of working in routinely processed and decalcified material. A high degree of sensitivity (80–95%) has been reported and the neuroblastic tumours have been negative. However, all the antibodies lack specificity as they mark a high percentage of T-cell lymphomas. Therefore caution must be exercised in the interpretation of immunoreactivity and it should be used in a panel of markers used for the immunocytochemical identification of SRCTs.

**Other tumours**

**Biphenotypic sarcomas:** Biphenotypic sarcomas show morphologic evidence of myogenic and neuroectodermal differentiation. A recent investigation demonstrated that they expressed the fusion gene associated with the Ewing family of tumours and not the products of the rearrangements associated with alveolar rhabdomyosarcoma.

**Desmoplastic small round cell tumour (DSRCT):** This is a distinct clinicopathologic entity which differs from the other childhood tumours
because of its clinical features, morphology and immunohistochemical features[^34]. Typically, DSRCTs occur in the adolescent males (male to female ratio 5:1, median age 18.6 years) and are located in the intra-abdominal, pelvic, retroperitoneal and scrotal sites. These are aggressive tumours with 90% mortality within 6 months to 4 years of diagnosis. Histologically, the DSRCTs show a nesting growth pattern (Fig. 3A) with an intense desmoplastic reaction while IHC reveals divergent differentiation with epithelial (Fig. 3B), myogenic (Fig. 3C) and neural markers (Fig. 3D)[^34]. A t(11;22)(p13;q12) has been consistently described in a limited number of these tumours. This involves the fusion of the EWS gene with the Wilms’ tumour 1 gene (WT1) which can be detected by molecular means for diagnostic purposes[^35].

### Brain tumours

The most common forms of brain tumour in children are primitive neuroectodermal tumours (PNETs) and low grade astrocytomas. PNETs of the central nervous system are not generally associated with the EWS gene rearrangements that are characteristic of the peripheral forms which belong to the Ewing family of tumours. An i(17q) is the most frequent cytogenetic finding in PNETs and in one study, 15 out of 34 cases were shown to have this rearrangement[^36]. The i(17q) is not unique to PNET but has been found as the sole cytogenetic anomaly in several cases. It has been suggested that the interphase FISH detection of the i(17q) could be of diagnostic use[^36]. Deletions of 17p involving the 17p13.3 containing a putative tumour suppressor gene region, distal to the TP53 locus, have been noted and in astrocytomas are associated with high grade tumours[^37].

The highly malignant rhabdoid tumours of the brain may be misclassified as PNET or medulloblastoma but, from the limited number karyotyped, they appear to be cytogenetically distinct with monosomy for chromosome 22. Mutations in the TP53 tumour suppressor gene are rarely found in paediatric brain tumours in contrast to the adult situation. This difference may contribute to the overall better prognosis and response to therapy in children[^38]. Patients with astrocytoma that have apparently normal karyotypes or nonclonal aberrations have been shown to have a significantly longer survival than those with clonal abnormalities, irrespective of the age of onset or grade of the tumour[^39]. However, the cytogenetic abnormalities found in paediatric astrocytomas are generally different from adult cases and further studies are required to correlate individual aberrations with a particular clinical phenotype.
Fig. 3 Desmoplastic small round cell tumours (DSRCT). (A) Nests of tumour cells set in a desmoplastic stroma. (B) Tumour cells showing epithelial (cytokeratin) immunoreactivity. (C) Dot-like cytoplasmic immunoreactivity to desmin. (D) Neuron-specific enolase immunoreactivity in tumour cells.
**Germ cell tumours (GCT)**

More than 80% of adolescent and adult testicular germ cell tumours are associated with an i(12p) chromosome and i(12p) negative germ cell tumours that have been investigated by FISH have an increased copy number of the whole or parts of the chromosome arm 12p. Although the underlying molecular mechanisms are not yet understood, the clinical value of detecting the i(12p) to establish the likely germ cell origin of tumours found in extra gonadal sites, particularly undifferentiated tumours in the mediastinum, is now well accepted. In contrast to adolescent cases, paediatric GCT are not generally associated with i(12p) formation although gain of 12p material is found. Other recurrent changes include loss of 1p material similar to that identified in neuroblastoma. The extent and potential prognostic significance of this loss in paediatric GCT requires further investigation.

**Anaplastic large cell lymphoma (ALCL)**

This type of non-Hodgkin’s malignant lymphoma is being increasingly recognised in children. ALCL is composed of large anaplastic cells (Fig. 4A) and histologically it has been misdiagnosed as malignant histiocytosis or lymphocyte-depleted Hodgkin's disease. Typically ALCL shows male predilection with an age range of 3–15 years and occurs in extranodal sites such as skin and lung. Immunohistochemically, the majority of ALCL cells display CD30 (Ki-1 antigen) (Fig. 4B) and are of a T-cell phenotype.

The chromosomal translocation t(2;5)(p23;q35) is observed in ALCLs. A novel chimeric protein p80\textsubscript{NMM/ALK1} results from the fusion of the ALK and NPM genes at 2p23 and 5q35, respectively. A monoclonal antibody which recognises this protein in frozen and routinely processed material was used for the immunohistochemical identification of ALCL with t(2;5). 30 of 105 ALCLs were positive for p80. Interestingly, not only were the majority of the p80 positive ALCLs children or adolescents, but p80 positive group also showed a statistically significant increase in 5 year survival in comparison to the p80 negative ALCL (79.8 vs 32.9%).

**Key points for clinical practice**

The aim of tumour classification is to group tumours in a way that has clinical value. The recent advances described indicate and offer a means
to identify the underlying molecular changes associated with different groups of paediatric tumours which can present a diagnostic problem using traditional immunocytochemistry and electron microscopy. In addition, some of the genotypic and phenotypic characteristics presented influence the clinical behaviour of the tumour. These are summarised in Table 1. The findings suggest that appropriate genotypic and phenotypic analysis should form an integral part of the laboratory assessment of such paediatric patients and impact on their clinical management. Further molecular characterisation may lead to additional diagnostic and prognostic indicators and potentially novel forms of treatment. Molecular characterisation in conjunction with monitoring patient
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progress, should lead to defining the most appropriate treatment for individual tumours and should improve patient management and survival.

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