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Commentary

Diagnosis and Classification of the Small Round-Cell Tumors of Childhood

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The small-round-cell tumors of childhood include neuroblastoma, the Ewing family of tumors, rhabdomyosarcoma, lymphoma, and desmoplastic small-round-cell tumor. Although classical histological features are generally highly suggestive of tumor type, on occasion these tumors may be indistinguishable by light microscopy, making a definitive diagnosis difficult. Accurate diagnosis of pediatric small-round-cell tumors has become increasingly crucial, as disparate approaches to therapy are used for distinct tumor types. In addition, because for many pediatric cancers, therapy is also tailored according to patient risk, it has become important to further classify tumors biologically, using cytogenetic or molecular studies to identify chromosome translocations, gene amplification, gene expression patterns, and/or mutations.

In this issue of *The American Journal of Pathology*, Gilbert and colleagues used a reverse transcriptase polymerase chain reaction (RT-PCR) assay to analyze the expression of two genes involved in the catecholamine biosynthetic pathway, tyrosine hydoxylase and dopa decarboxylase, in 84 pediatric malignancies.[1] Their studies demonstrate that the expression of these two genes is highly specific for neuroblastoma. Of the 29 non-neuroblastoma tumor samples examined, only pheochromocytomas expressed clearly detectable levels of the genes. These results suggest that analysis of tyrosine hydoxylase and dopa decarboxylase expression may help distinguish neuroblastoma from other small-round-cell childhood tumors.

Despite recent advances in immunohistochemistry and molecular pathology, some cases of small-round-cell tumors of childhood remain diagnostically problematic. Thus, additional diagnostic tools, such as the ones described by Gilbert and co-workers, are needed to ensure that every child with a small-round-cell tumor is diagnosed correctly. The value and limitations of current immunohistochemical, cytogenetic, and molecular studies as diagnostic aids for the small-round-cell tumors of childhood are highlighted below.

Immunohistology

Immunhistochemistry can be helpful in narrowing the differential diagnosis of small-round-cell tumors. For example, the cell surface glycoprotein p30/32^{MIC2} is highly expressed in the Ewing family of tumors.[2] Several monoclonal antibodies have been developed that detect different epitopes of this antigen. Many studies have used HBA71 or O13, and up to 98% of Ewing family tumors have been shown to exhibit immunoreactivity.[3,4] However, positive results with HBA71 can also be seen in non-neoplastic tissues and other tumor types, including rhabdomyosarcoma and non-Hodgkin's lymphoma.[5] Antibodies to desmin can be used to distinguish rhabdomyosarcoma from Ewing's sarcoma, neuroblastoma, and lymphoma.[6,7] Similarly, antibodies to leukocyte common antigen (LCA) can be used to separate hematolymphoid malignancies from the remainder of small-round-cell tumors.[8] Nevertheless, there is no antibody specific for a single tumor type. Overlap of mesenchymal, epithelial, and neural markers are present in a variety of tumors. Furthermore, reactivity to antibodies can vary depending on the preparation of the specimen, the antibody used, and the degree of tumor differentiation.

The immunohistochemical markers used for neuroblastoma have significant limitations. Neuron-specific enolase (NSE) is seen in neuroblastomas as well as tumors from the Ewing's family. In addition, NSE is present in a wide variety of non-neural cells, such as smooth muscle cells, and is present in some rhabdomyosarcomas.[6,9,10] Ganglioside 2 antibodies identify neuroblastomas but may also react in osteosarcoma and rhabdomyosarcoma.[11] Similarly, synaptophysin and neurofilament proteins are not specific for neuroblastoma.

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Cytogenetic and Molecular Studies

Cytogenetics are routinely performed in hematolymphoid malignancies, and in some cases the diagnosis is dependent on the presence of specific cytogenetic abnormalities. Similarly, many small-round-cell tumors of childhood also exhibit highly characteristic cytogenetic abnormalities. However, accurate karyotyping of solid tumors is technically difficult, and successful cytogenetic analysis can be performed in only a subset of cases. Despite the technical limitations, detection of a cytogenetic abnormality can be an important diagnostic aid in some childhood cancers. For example, the t(11;22)(q24;q12) translocation is frequently seen in the Ewing family of tumors, which includes Ewing's sarcoma, peripheral neuroectodermal tumors (PNET), and Askin's tumor.[12] However, this translocation is not specific for the Ewing's family of tumors. Recent studies have demonstrated that the t(11;22)(q24;q12) translocation can be identified in some cases of neuroblastoma[13] and rhabdomyosarcoma.[14]

Molecular approaches, including fluorescence *in situ* hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR), have facilitated the detection of chromosome translocations and have provided the methodology necessary for fully characterizing the involved genes. The t(11;22)(q24;q12) translocation has been shown to result in the production of a chimeric gene between *EWS*, which codes for a novel putative RNA-binding protein, and *FLI1*, a member of the *ETS* family of transcription factors.[15] Two other variant translocations have been described that involve other members of the *ETS* gene family. The t(21;22)(q12;q12) translocation involves the gene *ERG*, which is located on chromosome 21, and t(7;22)(p22;q12) translocation involves a gene known as *ETV1* at 7p22. Although several subtly different *EWS/FLI1* and *EWS/ERG* fusion transcripts have been identified,[16] to date, significant correlations between the different chimeric EWS transcripts and clinical parameters have not been identified.[17,18]

Specific cytogenetic abnormalities have also been used in the diagnosis and classification of rhabdomyosarcomas.[19] The translocation t(2;13)(q35;q14) is consistently found in the alveolar form of rhabdomyosarcoma.[20] This translocation results in the fusion of 5′ sequences of the *PAX3* gene to 3′ sequences of the gene *FKHR. PAX3* codes for a developmentally regulated transcription factor involved in muscle development[21] and *FKHR* is a member of the fork head family of transcription factors.[22] The PAX3-FKHR fusion protein has been shown to be a more potent transcriptional activator than PAX3 protein alone.[23] A variant t(1;13)(p36;q14) translocation has also been described in alveolar rhabdomyosarcoma, involving the *PAX7* gene located on chromosome 1.[24] Although the more common embryonal form of rhabdomyosarcoma does not exhibit a consistent cytogenetic profile, in many cases loss of heterozygosity on the short arm of chromosome 11, at 11p15.5, is found.[19]

Intra-abdominal desmoplastic small-round-cell tumor is an extremely rare, highly aggressive neoplasm that exhibits a nesting growth pattern.[25] Although only a few karyotypic studies have been performed on desmoplastic small-round-cell tumors, the reciprocal translocation t(11;22)(p13;q12) has consistently been described.[26,27] This translocation results in the creation of a fusion gene between the *EWS* gene and the Wilms tumor 1 gene (*WT1*).[28]

There are no consistent molecular genetic abnormalities in neuroblastoma tumors. However, cytogenetic abnormalities have been identified in subsets of neuroblastomas and found to be prognostically significant. For example, poor outcome is associated with deletion of the short arm of chromosome 1, 17q gain, and amplification of the *MYCN* oncogene.[29] Chromosome number or ploidy has also been shown to be clinically important in neuroblastoma. Hyperdiploidy is correlated with favorable outcome in patients with neuroblastoma, whereas resistance to chemotherapeutic agents has been observed in infants with diploid tumors.[30–34] In an effort to tailor therapy according to patient risk, Bowman and colleagues recently conducted a prospective nonrandomized Pediatric Oncology Group (POG) study using ploidy as the sole guide for treatment selection in infants with unresectable or metastatic neuroblastoma.[35] Patients with hyperdiploid tumors were treated with a less intensive chemotherapeutic regimen than those with diploid neuroblastomas. The 3-year survival estimate for 127 assessable infants with hyperdiploid tumors was 94%, whereas the overall 3-year survival estimate for the 41 infants with diploid disease was 55%. Although the outcome for patients with diploid tumors improved in this clinical trial compared with a previous study in which infants were treated with cyclophosphamide and adriamycin,[31] better therapy is still needed for this subset of patients.

Gene amplification generally is detectable by cytogenetic analysis either as extrachromosomal double minute chromatid bodies (DMs) or as chromosomally integrated homogeneously staining regions (HSRs).[36] DMs or HSRs are present in most neuroblastoma cell lines as well as some neuroblastoma primary tumors.[37–39] *In situ* hybridization studies have demonstrated that the normal single copy of *MYCN* is located on chromosome 2p24 and that in neuroblastoma *MYCN* amplification is present in the majority of DMs and HSRs.[40,41] However, *MYCN* amplification is not unique to neuroblastoma. Amplification of the oncogene has also been reported in some cases of rhabdomyosarcoma[42] and retinoblastoma.[43] Furthermore, high levels of *MYCN* expression have been detected in Wilms tumor and hepatoblastoma.[44]

MYCN amplification is seen in 30% to 50% of patients with advanced-stage neuroblastoma. In this subset of patients, *MYCN* amplification is strongly correlated with rapid tumor progression and poor outcome.[45,46] Only 5% to 10% of patients with localized disease or stage 4s neuroblastoma have tumors with *MYCN* amplification,[45,47] and the clinical relevance of *MYCN* amplification in favorable stage disease remains controversial.[47–49] There are reports of small numbers of patients with localized *MYCN*-amplified tumors treated with either surgery alone or surgery and low-dose chemotherapy that have been cured of their disease.[47,48] Furthermore, in a large Italian study, Tonini and colleagues recently reported that *MYCN* amplification was not associated with a worse

outcome in infants with stage 4s disease.[50] In contrast, poor outcome was seen in stage 4s infants with *MYCN* amplification in a study conducted by the POG.[51] The conflicting results highlight the biological heterogeneity of neuroblastoma, and suggest that multiple factors are likely to contribute to tumor phenotype.

Deletion of the short arm of chromosome 1 is found in ~30% of primary human neuroblastomas resulting from simple terminal deletions, interstitial deletions, and unbalanced translocations with known or unknown chromosome fragments to the short arm of chromosome 1.[37,38,52,53] It has been hypothesized that a neuroblastoma suppressor gene is located at 1p36, and this hypothesis is supported by the observation that neuroblastoma has developed in children with constitutional 1p abnormalities.[54,55] However, Maris and colleagues were unable to detect loss of heterozygosity at 1p36 in 13 patients with familial neuroblastoma, suggesting that there may be more than one NB suppressor gene.[56] Chromosome 1p deletions are most often seen in tumors that are near-diploid and *MYCN* amplified.[37,53,57–60] Two large independent studies have shown that while deletion of 1p is associated with unfavorable outcome in univariate analysis, this factor is not prognostic after adjusting for *MYCN* copy number.[58,61] In contrast, Caron and colleagues recently reported that loss of 1p was predictive of unfavorable outcome, independent of *MYCN* amplification.[60] Studies to identify the putative neuroblastoma suppressor gene (or genes) are ongoing.

Chromosome 17q abnormalities are also found in a subset of neuroblastoma tumors. Gilbert and co-workers found 17q gains in 8 of 35 (23%) neuroblastomas by classical cytogenetic technique.[39] Chromosome 17q abnormalities were subsequently detected by FISH and allelic imbalance studies by other investigators.[60,62,63] These studies demonstrated 17q gains in more than 90% of high-risk patients with neuroblastoma.[63] Clinical studies have demonstrated that favorable outcome is associated with whole chromosome 17 gains, whereas poor outcome is seen in the subset of neuroblastoma patients with 17q gains.[64]

The Trk family of neurotropin receptors are important regulators of survival, growth, and differentiation of normal and neoplastic cells, and there is increasing evidence that these genes also play an important role in the biology and clinical behavior of neuroblastoma tumors. Several independent retrospective studies have demonstrated that high levels of *TrkA* expression in neuroblastoma are associated with favorable outcome.[65–69] High levels of *TrkA* mRNA are present in tumors from patients with favorable stage disease, whereas low to undetectable levels are observed in *MYCN*-amplified tumors. Recently, high levels of expression of *TrkC* have also been shown to correlate with favorable prognosis in neuroblastoma patients.[70,71] Truncated *TrkB* is predominantly expressed in differentiated tumors, whereas co-expression of full-length *TrkB* and brain-derived neurotrophic factor (BDNF) is associated with *MYCN* amplification and may represent an autocrine survival pathway.[69,72]

Summary

Accurate diagnosis and classification of small-round-cell tumors of childhood has become increasingly important as modern therapy is not only disease specific but is also tailored according to patient risk. Despite advances in immunohistochemistry, cytogenetics, and molecular techniques, in some cases of small-round-cell tumors of childhood the correct diagnosis can remain elusive. Antibodies used in immunocytochemistry studies have limitations in sensitivity and specificity. Furthermore, although chromosomal abnormalities have proven to be useful in the characterization of certain pediatric cancers, other tumors lack a consistent genetic profile. It has also become evident that many genetic abnormalities are not tumor specific. Thus, although individual molecular tests can aid in delineating the entities of small-round-cell tumors of childhood, the diagnosis should not be based solely on the result of a molecular study. Rather, standard clinical and laboratory diagnostic modalities should be combined with immunohistochemistry, cytogenetics, and molecular studies. Studies similar to the one reported by Gilbert and colleagues,[1] analyzing the expression of patterns of genes specific to certain tumor types, are likely to result in the identification of additional molecular tools that will ensure that each child with a small-round-cell tumor is diagnosed correctly. The identification of additional molecular markers may also lead to a further refinement of risk-group classification and thereby provide the biological information needed to determine optimal treatment for every affected child.

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