

Constellations of Genetic Abnormalities Predict Clinical Outcome in Childhood Malignancies *

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A. Introduction

A major thrust of tumor classification systems has been to recognize clinical and histological differences among tumors arising from the same or similar tissues, in the belief that such distinctions will provide a framework for the development of improved treatment. Clearly, this approach has been instrumental in advances toward uniformly curative treatment. However, the variable therapeutic responsiveness of many histopathologically classified tumors suggests the presence of biologically unique subgroups with prognostic importance. The challenge confronting pediatric oncologists is to devise classification schemes that will accommodate the biological diversity of childhood tumors. We need to be able to recognize, at diagnosis, those patients who will respond well to therapy despite having high-risk features by conventional criteria. Within so-called good-risk groups, we need to identify patients whose tumors have exceptional sensitivity to standard therapy, so that the severe acute toxicity and adverse late effects associated with intensive treatments can be avoided. Conversely, we need to recognize with greater reliability all exception-

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ally high risk patients for whom standard treatment approaches are likely to be futile.

The thesis of this article is that recognition of constellations of closely related genetic features may provide the predictive edge in treatment planning. Genetic abnormalities of tumor cells are in general acquired as somatic events and are probably tightly linked to the processes of malignant transformation and clonal evolution of aggressive growth properties within the malignant clone. Our studies indicate that certain profiles of genetic markers identify prognostically relevant subgroups within histopathologically defined childhood neoplasms. These genetic abnormalities include alterations of the ploidy (chromosome number) of malignant clones and the presence of structural cytogenetic abnormalities, such as translocations or deletions, that appear specific for subgroups of tumors. At the molecular level, gene amplification, rearrangement, mutation, and deletion indicate somatically acquired alterations that bear directly on the biological behavior of tumors. Because genetic abnormalities occur in defined patterns in human tumors, simple laboratory tests, such as the detection of abnormalities of ploidy by DNA flow cytometry, have proved especially useful for the prediction of prognosis in the clinical setting. My intention in this review is to present and discuss our findings in four different childhood neoplasms – unresectable neuroblastoma of infancy, acute lymphoblastic leukemia, Wilms' tumor, and osteogenic sarcoma of an extremity – to illustrate how discrete patterns of genetic abnormalities can be used as guides to optimal therapy.

B. Disseminated Neuroblastoma of Infancy

We have shown that the ploidy of neuroblasts from infants with unresectable neuroblastoma, as detected at diagnosis by flow cytometric analysis of cellular DNA content, is closely linked to prognosis [1]. Although approximately 70% of infants with metastatic neuroblastoma are cured with five courses of cyclophosphamide and doxorubicin, the remaining 30% do not achieve a lasting response [2]. Infants whose tumors have diploid cellular DNA content are the ones who fail to respond to this therapy, while those with clonal hyperdiploid tumors have the best responses and are almost always cured of their disease [1, 3]. Our initial observations have now been extended to a total of 61 infants with unresectable disease who were treated with five courses of cyclophosphamide plus doxorubicin. In that study, conducted under the aegis of the Pediatric Oncology Group, approximately 90% of infants with hyperdiploid tumors responded to therapy, and approximately 80% have achieved durable remissions and are apparently cured. By contrast, of infants with diploid tumors, 60% failed to show an appreciable response to therapy, and only about 20% of this group are long-term disease-free survivors.

Comparison of ploidy findings with the presence or absence of *N-myc* gene amplification (determined by Dr. Garrett Brodeur) and structural karyotypic alterations (determined by Dr. Edwin C. Douglass) indicated that in neuroblastoma of infancy, diploid tumors are the ones with high levels of *N-myc* amplification [4–7] and abnormalities of chromosome structure, such as deletions of the short arm of chromosome 1 [8–11]. By contrast, hyperdiploid tumors tend to have simple additions of chromosomes with single copy numbers of the *N-myc* gene and no abnormalities of chromosome structure. The value of flow cytometry for classification of neuroblastoma in infants lies in its ability to serve

as a marker for tumors with clinically relevant constellations of genetic abnormalities (Table 1). Whether any of the changes identified so far contribute directly to the observed patterns of drug sensitivity is unclear. It may be that the constellations we have recognized include other, as yet unidentified, genetic alterations that in fact mediate cellular sensitivity to chemotherapy. Whatever the explanation, it is now possible to use genetic measurements of neuroblastoma of infants to reliably predict response to therapy.

The above findings provided the impetus for a prospective clinical trial (conducted by Dr. Ann Hayes within the Pediatric Oncology Group) in which therapy for infants with neuroblastoma is being modified according to the patient's pretreatment DNA index. At the time of diagnosis, tumor cells are submitted for evaluation of DNA content, and patients are treated with one course of cyclophosphamide plus doxorubicin. The DNA index, defined as the ratio of the modal DNA content of G_0/G_1 -phase tumor versus normal cells, is used to determine whether the patient will be given four additional courses of the drug combination (hyperdiploid tumors) or whether the therapy will be switched to platinum plus VM-26 (diploid tumors). The alternative treatment with platinum and VM-26 is both more effective and more toxic than therapy with cyclophosphamide and doxorubicin. In previous studies, the combination was shown to induce temporary remissions in approximately half of all infants who failed therapy with first-line agents [12]. If successful, this strategy should yield longer remissions in infants with high-risk genetic features while sparing others the added toxicity produced by cisplatin and VM-26.

We think it interesting that the well-defined genetic constellations in tumors of infants are not found in neuroblastomas in children over 1 year of age. *N-myc* gene amplification and structural abnormalities of chromosome 1 occur with equal frequency in tumors with diploid

Table 1. Constellations of clinically relevant genetic abnormalities in three childhood malignancies

Tumor	Genetic markers	
	Favorable prognosis	Unfavorable prognosis
Unresectable neuroblastoma in infants	Hyperdiploidy (DNA index > 1) Absence of chromosomal structural changes or DMs/HSRs Single copy of <i>N-myc</i> gene	Near diploidy (DNA index = 1) Chromosome 1p deletions; DMS, or HSRs <i>N-myc</i> gene amplification
Acute lymphoblastic leukemia	Hyperdiploidy ≥ 53 chromosomes (DNA index ≥ 1.16) Simple additions of chromosomes with few translocations Trisomes preferentially involving chromosomes 4, 6, 10, 14, 17, 18, 20, 21 and X	Near diploidy (DNA index = 1) Specific chromosomal translocations [t(8;14), t(9;22), t(4;11)] <i>bcr-abl</i> fusion genes resulting from t(9;22)
Wilms' tumor	Near diploidy (DNA index = 1.0) Lack of complex chromosomal translocations	Near tetraploidy (DNA index = 2.0) Multiple complex chromosomal translocations

or hyperdiploid DNA content. Moreover, a group of older children with disseminated neuroblastoma and a potentially favorable outcome cannot be identified with any of these genetic markers, because the disease is almost uniformly fatal despite aggressive combination chemotherapy. New approaches to therapy are urgently needed for essentially all children more than a year of age who have disseminated disease at diagnosis.

C. Childhood Acute Lymphoblastic Leukemia

Improvements in the treatment of children with acute lymphoblastic leukemia (ALL) have galvanized efforts to identify features of leukemic cells that will reliably predict the clinical course of this disease in individual patients. It is important to identify patients who have a high risk of treatment failure and who would benefit from very intensified programs of therapy [13]. Concern over the immediate and long-term adverse sequelae of intensive chemotherapy, however, has stimulated attempts to identify children who respond well to less intensive treat-

ment, so that they can be spared unnecessary toxicity.

It has been recognized for years that children with greater than 50 chromosomes in their leukemic cell karyotype have a more favorable prognosis than those with lower leukemia cell ploidies [14–19]. Careful analysis of the karyotypes of leukemic blasts with greater than 50 chromosomes has defined a constellation of cytogenetic features, such as trisomies of specific chromosomes and a relatively low frequency of translocations [19] (Table 1). About 20% of children have ploidy values in this range, and they comprise a subset of a larger common ALL antigen-positive group characterized by pre-B or B-cell precursor phenotypes [20]. We have shown that flow cytometric measurement of the DNA content of leukemic blast cells, expressed as a DNA index, is the method of choice for identifying patients in the hyperdiploid group who have a favorable prognosis. In a recent analysis of flow cytometric results for patients treated in St. Jude Total Therapy Study X, we found that the most favorable prognostic feature was a leukemic cell DNA index greater than or equal to 1.16, which corresponds to

greater than or equal to 53 chromosomes per leukemic cell [21, 22]. Interestingly, the best results were obtained in the chemotherapy arm in which high-dose intravenous methotrexate plus intrathecal methotrexate was used for both intensification and central nervous system prophylaxis. The remainder of the treatment program included induction therapy with prednisone, L-asparaginase, and vincristine, and continuation therapy with mercaptopurine and low-dose methotrexate. With this treatment, an estimated 89% of children whose blasts had a DNA index greater than or equal to 1.16 will be in continuous remission for 3 years and 82% for 5 years. The overall treatment program is remarkable because of its low short-term toxicity and its reduced potential for producing adverse late effects, compared with other protocols that employ cranial irradiation and mutagenic agents such as cyclophosphamide and anthracyclines.

A group of patients with a much higher risk of treatment failure can also be defined by cytogenetic analysis (Table 1). These patients tend to have near-diploid chromosome complements in their leukemic blasts and an increased frequency of chromosomal translocations associated with a high risk of treatment failure, such as the t(8;14), t(9;22), and t(4;11) [19, 23]. Molecular genetic correlates of these specific translocations will undoubtedly be of increasing importance in the understanding of the pathogenesis of ALL and in the design of optimal therapy for this disease. For example, the t(9;22) (q34;q11) found in chronic myelogenous leukemia and some cases of ALL results in translocations of the *c-abl* gene from chromosome 9 to chromosome 22 [24–26]. The breakpoint on chromosome 22 in CML disrupts a gene called *bcr* (breakpoint cluster region) within a well-defined 5.8-kb region of genomic DNA [27–29]. Translocation results in fusion of the *bcr* and *c-abl* genes with production of a characteristic 8.5-kb mRNA [30–35] and a 210-kb hybrid protein that is activated as a tyrosine-

specific kinase [36–39]. Although the 9;22 translocation that gives rise to the Philadelphia chromosome is cytogenetically identical in ALL to that found in CML, molecular studies have now revealed a potentially important difference. In ALL cells that harbor the Philadelphia chromosome, a 6.5- to 7.0-kb fusion transcript and a 185- to 190-kd hybrid protein are produced that are distinct from those of both the normal *c-abl* gene and the rearranged *bcr/c-abl* fusion gene found in CML [40–42]. The breakpoints of chromosome 22 in ALL cases with the 9;22 are not within the 5.8-kb region of *bcr* that contains the breakpoints in CML, but lie further upstream within the *bcr* gene. The ALL fusion protein includes aminoterminal determinants of the *bcr* gene but lacks internal *bcr* determinants that are found in the CML fusion protein near the *bcr-abl* junction [43–45]. It is now possible with molecular probes to detect rearrangements within the *bcr* fragment that is characteristically affected in CML, and it appears likely that probes will soon be defined that can identify the ALL abnormality. The molecular characterization of chromosomal translocations found in ALL promises to improve the understanding of the molecular basis of this disease, and to provide new means of identifying genetic rearrangements that have very specific implications for therapy.

D. Wilms' Tumor

In Wilms' tumor, the majority of patients have tumors with near diploid DNA content and karyotypes that lack major chromosomal rearrangements or translocations [46]. These patients also have favorable histological features, and a very high probability of cure with current treatment modalities. By contrast, a constellation of genetic features including cellular DNA content in the near-tetraploid range and multiple complex chromosomal translocations defines a group with anaplastic histological features and

a very unfavorable treatment response (Table 1). As is the case in neuroblastoma and acute lymphoblastic leukemia, cellular DNA content measurements by flow cytometry appear to be the most practical means of identifying high-risk tumors in the clinical setting. It is important to note, however, that the correlation between very hyperdiploid tumors and a poor response to treatment is exactly opposite that observed in neuroblastoma in infancy and acute lymphoblastic leukemia. This emphasizes the fact that tumor-cell ploidy is probably not a primary determinant of drug responsiveness. Rather, it appears to be a marker of subgroups of tumors with unique cytogenetic and molecular genetic features. Thus, the correlation observed in Wilms' tumor between very high ploidy levels and complex chromosomal translocations may indicate that in this disease, high ploidy levels are associated with "genetic instability," which results in an increased rate of mutations leading to drug resistance. By contrast, in neuroblastoma of infants and acute lymphoblastic leukemia, hyperdiploid tumor cells tend to have the addition of whole chromosomes without structural rearrangements and thus may be less likely to have key genetic lesions, despite their higher ploidy levels.

E. Osteosarcoma of an Extremity

We have recently characterized the cellular DNA content of osteosarcoma cells obtained at diagnosis from patients with extremity lesions who lacked clinically evident metastases [47]. When treated with intensive combination chemotherapy as part of a large multi-institutional trial, patients with near-diploid tumor stem lines had a markedly improved disease-free survival by comparison with those whose tumors had only hyperdiploid stemlines. Thus, in this disease as in Wilms' tumor, hyperdiploidy correlated with an adverse prognosis. One important difference was observed, however. For osteosarcoma, the favorable in-

fluence of near diploidy was apparent even if additional hyperdiploid lines were present. Thus, in osteosarcoma there may be an interaction between stem lines of different ploidies that we have not observed in other tumors, possibly indicating that in osteosarcoma near-diploid stemlines may provide growth factors that are required by the hyperdiploid stem lines that accompany them.

F. Concluding Remarks

Our results indicate that for several types of childhood malignancies, it is possible to identify constellations of genetic abnormalities that appear important in pathogenesis and provide remarkable correlates with drug sensitivity. A goal of our studies has been to use such abnormalities to improve the design of clinical trials, which means that detection must be practical at the time of diagnosis for every patient's tumor in a clinical setting. One of the advantages of using flow cytometry to measure cellular DNA content is that reliable results can be obtained with samples shipped from participating institutions to a reference laboratory. Fortunately, it appears that simple measurements of tumor-cell ploidy can be used to identify subsets of patients with defined patterns of genetic abnormalities, including those that influence the responsiveness of tumor cells to chemotherapy. Careful clinical studies are needed to define important relationships between genetic abnormalities and therapeutic responsiveness for each tumor type. A prediction of these studies is that as molecular genetic abnormalities are more completely characterized in human tumors, the prognostic importance of these alterations will be increasingly useful for the design of optimal treatment programs.

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