Hedgehogs, Flies, Wnts and MYCs: The Time Has Come for Many Things in Medulloblastoma

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Medulloblastoma epitomizes the war against childhood cancer. Although this small round blue-cell tumor accounts for only 20% of all childhood brain tumors, or just fewer than 600 new cases each year in the United States,1 medulloblastoma has nevertheless become a paradigm for biologic study, clinical investigation, and assessment of late effects. Craniospinal irradiation historically became the mainstay of therapy for this WHO grade 4 neoplasm because of its propensity to disseminate throughout the neuraxis via the subarachnoid space. By the 1970s, 36 Gy of craniospinal radiotherapy resulted in 5-year survival of greater than 50% for better-risk patients.7 With cooperative group trials in the 1980s, adjuvant chemotherapy was introduced to medulloblastoma treatment protocols (SIOP I [International Society of Paediatric Oncology], CCG 942 [Children’s Cancer Group], and POG 7909 [Pediatric Oncology Group]), leading to improved survival and subsequent reduction of radiotherapy dosage for average-risk patients6,7 in the 1990s and early part of the last decade. A decrease in craniospinal radiotherapy from 36 Gy to 23.4 Gy resulted in better neurocognitive and physical outcomes for average-risk patients,8 highlighting the importance of developing appropriately risk-adapted therapy.

Although prognosis has improved over many years, a significant fraction of children still do not respond to standard therapy. Perhaps equally disturbing, survivors are left with a multitude of sequelae attributable to chemotherapy and radiation therapy, including cognitive impairment, hearing loss, neuropathies, seizures, endocrinopathies, strokes, and second malignancies.9,10 Thus, there is an overwhelming need to refine strategies that will minimize unnecessarily aggressive treatment for patients with low-risk disease and maximize efficacious therapy for patients with high-risk disease. Such refinement can come only with better disease or risk classification.

In an era of genomic profiling, risk stratification for medulloblastoma disappointing remains strictly clinical, based solely on age, resection extent, Chang metastasis staging, and more recently anaplasia.11 Since the mid 1990s, a two-tiered risk stratification system has been employed for the purposes of North American cooperative group trials. Patients older than 3 years with less than 1.5 cm² of residual tumor and nonmetastatic disease are considered average risk, whereas those younger than 3 years with more than 1.5 cm² of residual tumor, evidence of tumor dissemination, or diffuse anaplasia are considered high risk.

Multiple histopathologic, cytogenetic, and molecular factors have been found to influence prognosis, but to date, none have been incorporated into the risk-stratification system. Histopathologically, the desmoplastic variant has long been recognized as connoting better prognosis,12 whereas the large-cell anaplastic variant more recently has been found to be associated with reduced survival.13-15 Moreover, isochromosome 17q, loss of heterozygosity at chromosome 17p,16 and high-level chromosomal gain at 8q24 (locus of c-MYC) have all been associated with large-cell anaplastic variant medulloblastoma17 and poor prognosis. Monosomy 6 has been associated with favorable prognosis.18-20 In addition, a number of molecular markers have been identified over the past decade and have laid the groundwork for a risk-stratification system with a biologic basis. Favorable prognosis has been described for children with high TrkC expression21,22 or nucleopositivity of β-catenin, an indicator of activated Wnt signaling—a pathway now known to affect embryogenesis and cancer.18-20,23,24 Poor prognosis is indicated by overexpression of calbindin-D,25 elevated ErbB2 expression,16,26 and elevated expression of c-MYC, a member of the MYC family of transcription factors that stimulates cell proliferation.27-29

In recent years, large-scale genomic and gene expression profile studies have begun to parse the molecular heterogeneity of medulloblastomas into clear subtypes with associated risk stratification. Initial gene expression microarray studies used a supervised approach, grouping samples on the basis of histology, metastatic status, and survival to identify differentially expressed genes in these clinical and histologic groups.30 More recently, unsupervised microarray studies in medulloblastoma by Kool et al19 (62 samples) and Thompson et al24 (46 samples) each identified up to five subgroups of medulloblastoma, including a subgroup characterized by activation of the Wnt signaling pathway, associated with excellent prognosis, and a subgroup characterized by activation of the Hedgehog (Hh) signaling pathway occurring primarily in infants and adults and associated with desmoplastic histology. The Hh signaling pathway is involved in organogenesis, limb formation, brain patterning, and multiple forms of cancer. There are several Hh family ligands, the best known of which is Sonic Hedgehog (SHh) for its multiple developmental roles, including in cerebellar development. The study by Kool et al also identified a molecular subtype associated with a high rate of metastatic disease, anaplastic/large-cell histology, and poor prognosis.

In this issue of Journal of Clinical Oncology, four separate studies dovetail with previous work to crystallize a molecular classification of medulloblastoma and codify a new risk-stratification model on the basis of the molecular biology of these disease subtypes. Ellison et al31...
report the identification of low-, average-, and high-risk subtypes of medulloblastoma. This study used 207 medulloblastoma samples and employed immunohistochemistry, fluorescence in situ hybridization, and direct sequencing of DNA to evaluate for a Wnt pathway activation signature, c-MYC amplification, and copy-number abnormalities of chromosomes 6 and 17. Molecular data were correlated with histology and clinical outcome. Consistent with previous work, Wnt pathway activation and the associated monosomy 6 marked a subset of tumors with excellent prognosis. In contrast, c-MYC amplification was found in a subset of patients with poor event-free and overall survival (Table 1).

Cho et al32 describe six molecular subgroups of medulloblastoma on the basis of analysis of 194 samples for gene expression and DNA copy-number abnormalities. A subset of samples in this work was also analyzed for inhibitory mRNAs. Molecular findings were correlated with clinical outcome, and a novel probabilistic model, described in a companion article by Tamayo et al,33 integrates the molecular and clinical data to predict outcome in medulloblastoma on a case-by-case basis. The study by Cho et al similarly found Wnt pathway–active, Hh pathway–active and c-MYC–amplification subgroups associated with excellent, good, and poor prognoses, respectively (Table 1). The Hh group, categorized based on dysregulated activity of the Hh pathway, seemed to be linked more with males and had intermediate prognosis. The subgroup with amplified c-MYC exhibited expression of genes in phototransduction pathways. Of the three other subtypes described by Cho et al, one subgroup was characterized by expression of neuronal differentiation pathways but not photoreceptor pathways, and the other two subgroups exhibited gene expression patterns that overlapped with this latter group and with the c-MYC–amplification group.

The study by Northcott et al34 analyzed 103 medulloblastoma samples for gene expression patterns and genomic copy-number aberrations to establish four molecular subtypes of disease and correlate them with demographic data, histology, and clinical outcome (Table 1). Northcott et al describe a Wnt subtype associated with excellent prognosis, Hh subtype associated with good prognosis, c-MYC–amplification subtype associated with metastatic disease and poor prognosis, and fourth subtype associated with fair prognosis. It is worth noting that elevated expression of MYC family transcription factors (n-MYC and c-MYC) was found in the Wnt-, Hh-, and c-MYC–amplification subgroups (Table 1). Because MYC gene expression is a downstream target of Hh and Wnt signaling,35 this is not a surprise. How elevated MYC expression contributes to the different biologic behavior of these different subtypes of medulloblastoma is not yet clear. It may be the case that Hh and Wnt pathway activity elevate MYC only to physiologic levels, whereas MYC-amplified tumors express MYC at supraphysiologic levels, leading to the more aggressive phenotype of the MYC–amplification subgroup. Consistent with the studies by Cho et al32 and Kool et al,36 the c-MYC–amplification subgroup exhibited expression of genes associated with phototransduction and neuronal differentiation. The fourth subtype in the Northcott et al study also exhibited neuronal differentiation pathway gene expression. The authors then identified a specific molecular marker—a signature gene—for each subtype (Table 1) and used commercially available antibodies against these four markers suitable for immunohistochemistry in formalin-fixed paraffin-embedded samples. They validated this four-marker strategy in 294 medulloblastoma tissue samples, confirming the risk stratification observed in the gene-expression and copy-number analyses with the demographic and clinical data associated with these 294 formalin-fixed paraffin-embedded tissue samples. That a readily accessible and relatively inexpensive technique such as standard immunohistochemistry could determine medulloblastoma subtype is remarkable; this could allow for the rapid translation of a molecular medulloblastoma classification system into clinical practice.

What can we conclude? Lewis Carroll’s sapient walrus said, “The time has come to talk of many things: Of shoes—and ships—and sealing wax—and cabbages—and kings.”36(p185) Yes, in medulloblastoma, it is time to talk of many things: of Hhs, flies, Wnts, and MYCs. The evidence from multiple independent studies clearly points to the existence of at least four molecular subtypes of medulloblastoma with differential outcomes.19,24,30,32,34 The Wnt pathway–active subtype, associated with monosomy 6 and occurring in all age groups, has excellent prognosis. Interestingly, recent work indicates a distinct cellular origin outside the cerebellum for the Wnt subtype.37 The Hh pathway–active subtype, associated with desmoplastic histology and occurring primarily in infants and adults, has good prognosis. The

Table 1. Medulloblastoma Subtypes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Wnt</th>
<th>Hh</th>
<th>c-MYC Amplification</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prognosis</td>
<td>Excellent</td>
<td>Good</td>
<td>Dismal</td>
<td>Fair</td>
</tr>
<tr>
<td>Demographic</td>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male to female ratio</td>
<td>1:1.7</td>
<td>2:1</td>
<td>1:0.7</td>
<td>1:0.5</td>
</tr>
<tr>
<td>Frequency (all ages), %</td>
<td>10</td>
<td>33</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>Metastatic disease, %</td>
<td>0</td>
<td>7</td>
<td>75</td>
<td>31</td>
</tr>
<tr>
<td>Histologic subtype</td>
<td>Monosomy 6</td>
<td>Desmoplastic</td>
<td>Anaplastic/large cell</td>
<td></td>
</tr>
<tr>
<td>Genomic abnormality</td>
<td>Wnt pathway</td>
<td>Hh pathway</td>
<td>Neuronal differentiation pathways</td>
<td></td>
</tr>
<tr>
<td>Elevated gene expression</td>
<td>c-MYC</td>
<td>n-MYC</td>
<td>c-MYC</td>
<td></td>
</tr>
<tr>
<td>Immunomarker</td>
<td>DKK1</td>
<td>SFRP1</td>
<td>NPR3</td>
<td>KCNA1</td>
</tr>
</tbody>
</table>

Abbreviation: Hh, Hedgehog.
c-MYC–amplification subtype, associated with anaplastic/large-cell histology and occurring primarily between ages 3 to 10 years, carries dismal prognosis. The other subtypes seem to occur in all age groups and carry fair prognosis.

Where do we go from here? One might be tempted to refine these subgroups even further, because these classifications are imperfect. For example, the Hh pathway–active subgroup has some heterogeneity in age and genomic profile. However, patients waiting in the clinic right now are entitled to better risk stratification than that of the anachronistic system we are currently using. We should move forward quickly. First, the new molecular medulloblastoma classification and risk-stratification system indicated by these numerous complementary studies should be confirmed retrospectively using tissue samples and clinical outcome data from the recent large International Society of Pediatric Oncology (SIOP) trial (SIOP PNET–4: A Prospective Randomised Controlled Trial of Hyperfractionated Versus Conventionally Fractionated Radiotherapy in Standard Risk Medulloblas-toma) and Children’s Oncology Group (COG) trials (A9961: A Phase III Prospective Randomized Study of Craniospinal Radiotherapy Followed by One of Two Adjuvant Chemotherapy Regimens (CCNU, CDDP, VCR or CPM, CDDP, VCR) in Children With Newly Diagnosed Average-Risk Medulloblastoma; ACNS 0331: A Study Evaluating Limited Target Volume Boost Irradiation and Reduced Dose Craniospinal Radiotherapy (18.00 Gy) and Chemotherapy in Children With Newly Diagnosed Standard Risk Medulloblastoma: A Phase III Double Randomized Trial; ACNS 0332: Efficacy of Carboplatin Administered Concomitantly With Radiation and Isotretinoin as a Pro-Apoptotic Agent in Other Than Average Risk Medulloblastoma/PNET Patients; and ACNS 0334: A Phase III Randomized Trial for the Treatment of Newly Diagnosed Supratentorial PNET and High Risk Medulloblastoma in Children Less Than 36 Months Old With Intensive Induction Chemotherapy With Methotrexate Followed by Consolidation With Stem Cell Rescue Versus the Same Therapy Without Methotrexate) (17). Once expeditiously validated, the rapid implementation of a new system will allow for tailored trial design, aimed at minimizing treatment effects and maximizing cure rate. For instance, patients with the Wnt subtype are perhaps being overtreated, and their treatment-related sequelae could be minimized. Children in the c-MYC–amplification subgroup may require therapy more aggressive than the current standard of care. Moreover, patients in the Wnt and Hh pathway–active subgroups have clearly defined targets for specific therapy, because small-molecule pathway–inhibitor drugs already exist for the Hh pathway and are in development for the Wnt pathway, and trials can now be designed for specific pathway inhibitors with inclusion of only those patients with the appropriate disease subtype. Each subtype might be identified in routine clinical practice by something as simple as immunohistochemistry on the basis of single subtype–specific markers and other markers, and therapy might then be tailored to each. Rapid turnaround with simple inexpensive techniques will be essential if these subtypes are to be readily identified for tailored therapy.

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