Plasticity of Neuroblastoma Tumor Cells to Differentiate along a Fetal Adrenal Ganglionic Lineage Predicts for Improved Patient Survival

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Abstract

We have recently presented a model of human adrenal medullary histogenesis that incorporates all neural crest-derived lineages (chromaffin, sustentacular, and ganglionic) known to compose this tissue. To determine if neuroblastomas correspond to the arrested maturation of embryonal adrenal medullary cells, we evaluated the expression of adrenal medullary developmental markers in 81 neuroblastoma tumors. We found that patterns of chromaffin-related gene expression in these tumors correlated exactly with the patterns observed during maturation of adrenal medullary cells \( (P < 10^{-5}) \). In a multivariate Cox proportional hazards analysis of developmental marker expression and other well-recognized prognostic variables, evidence of maturation along a fetal ganglionic lineage, as monitored by HNK-1 immunoreactivity (relative risk of 6.42, \( P = 0.0001 \)), and age at diagnosis (relative risk of 5.05, \( P = 0.0042 \)) were independent and significant prognostic indicators of patient survival. These studies demonstrate that neuroblastomas correspond to embryonal adrenal medullary cells arrested at recognizable stages during development, and that evidence of maturation along a fetal ganglionic lineage appears to have major importance in predicting patient survival. (J. Clin. Invest. 1992. 90:2402-2408.) Key words: neuroblastoma • differentiation • neural crest • HNK-1 • prognostic marker

Introduction

Neuroblastoma, a pediatric tumor arising in tissues derived from the embryonal neural crest, most frequently occurs at known sites of the sympathetic peripheral nervous system (1). This tumor exhibits remarkable heterogeneity in its histopathologic appearance, biochemical properties, and response to chemotherapy (2–8). Although a number of prognostic indicators of patient survival have been described for this tumor, including age of the patient at diagnosis, stage, amplification of the N-myc oncogene, the Shimada histopathologic classification, expression of neuropeptide Y, and serum ferritin levels (6, 9–13), the biological basis for the significance of these prognostic factors remains poorly understood.

Clinical, histopathologic, and laboratory findings suggest that altered regulation of neural crest cell differentiation can play a role in the pathogenesis of neuroblastoma (14–18). For example, the remarkably high incidence of neuroblastoma in situ reported in autopsy series of infants dying from causes unrelated to cancer suggests that many of these in situ tumors spontaneously remit, perhaps by differentiating to mature adrenal medullary tissues (14, 15). Similarly, stage IVS neuroblastomas, a variant of stage IV disease presenting itself in patients < 1 yr of age who have small primary tumors and remote disease confined to liver, skin, and/or bone marrow, often undergo spontaneous tumor regression, occasionally resulting in residual, benign ganglioneuromas (19–21).

We therefore investigated the possibility that neuroblastoma tumor cells may correspond to the arrested differentiation of adrenal medullary progenitor cells found during histogenesis of this tissue. In previous studies, we identified and characterized a series of markers defining discrete stages during the maturation of adrenal medullary chromaffin cells and distinguishing cells of this lineage from other neural crest-derived adrenal precursors that differentiate along either the sustentacular or ganglionic lineage (17, 22, 23). We found that neuroblastoma cell lines express these chromaffin markers in patterns found during development, suggesting that these tumor cells correspond to adrenal medullary chromaffin progenitor cells that are arrested in differentiation (17). In this study, we examined the expression of these lineage-related markers in neuroblastoma tumor specimens and evaluated the clinical implications of such a developmental analysis.

Methods

Tumor specimens. 81 formalin-fixed, paraffin-embedded neuroblastoma tumor specimens obtained from Children's Hospital of Philadelphia were used in this study. These samples were 85% (81/95) of accrued tumor specimens in the Department of Pathology collected between 1972 and the beginning of 1987 that had associated complete clinical information. Most of these patients have been included in previous studies evaluating antineoplastic therapies and the prognostic importance of clinical and biochemical correlates of improved patient survival (9). While the patients participating in these clinical trials received a variety of diagnostic, therapeutic, and supportive interventions, the results of these studies did not demonstrate major differences in survival based on the therapeutic regimens used to treat these patients.

Immunohistochemical analysis. Serial 5-μm sections of tumor specimens were analyzed for tyrosine hydroxylase (TH)1, chromogranin A (CGA), beta2-microglobulin (B2M), S-100, HNK-1, and neuron-spe-

This study was presented in part at the 26th Meeting of the American Society of Clinical Oncology, Washington, DC, 20–22 May 1990, and was published in abstract form (1990. Proc. Am. Soc. Clin. Oncol. 9:293).

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Received for publication 24 March 1992 and in revised form 2 July 1992.
cific enolase immunoreactivity using an avidin-biotin complex, horse-
radish peroxidase-linked detection system (Vectastain ABC peroxidase
kit; Vector Labs, Inc., Burlingame, CA) as previously reported (17, 23).
To facilitate analysis of chromaffin and ganglionic markers in
discrete tumor cell populations, the section stained for HNK-1 immu-
noreactivity was adjacent to those stained for CGA and TH. In no case were we able to find cells that expressed markers of both
lineages. Primary antibodies included a rabbit anti-bovine tyrosine
hydroxylase antiserum (Eugene Tech International, Allendale, NJ)
used according to the manufacturer’s recommendations; a mouse IgG,
anti-human chromogranin A monoclonal antibody (clone LKH210)
used at a concentration of 10 μg/ml (Boehringer Mannheim Biochemi-
cals, Indianapolis, IN); a rabbit anti-human B2M antiserum (Dako Lab-
oratories, Carpinteria, CA) used at a dilution of 1:100; a rabbit anti-bo-
vine brain S-100 antiserum used at a dilution of 1:1,500 (Accurate Chem.
& Sci. Corp., Westbury, NY); HNK-1, a mouse IgM monoclonal anti-
body used at a dilution of 10 μg/ml (Becton Dickinson Immunocyto-
metry Systems, Mountain View, CA); and a rabbit anti-human neuron
specific enolase antiserum used at a dilution of 1:500 (Accurate Chem.
& Sci. Corp.). Control antibodies included isotype matched mouse anti-
mouse H2 antigen-specific monoclonal antibodies (provided by Dr.
David Sachs, National Institutes of Health, Bethesda, MD) and puri-
fied rabbit immunoglobulin (Accurate Chem. & Sci. Corp.). Diamino-
benzidine (0.05%) (Sigma Chemical Co., St. Louis, MO) was used as the
chromogen, and the specimens were counterstained with hematox-
ylin and eosin using standard techniques. Specimens were examined for
marker expression and given final assignments before obtaining
survival data. A tumor specimen was scored as positive for a marker if
> 5% of tumor cells in multiple high power fields were immunoreac-
tive. Tumor specimens were categorized as “null” if they lacked any
lineage-related marker expression. Nevertheless, all tumor samples, in-
cluding those in the null category, expressed the nervous system
marker, neuron-specific enolase.

Statistical methods: Correlations between the expression of line-
age-related markers and prognostic indicators of neuroblastoma pa-
tient survival were determined separately; lineage assignment was eval-
uated for each prognostic variable using the chi-square test. Because 16
comparisons were involved in this analysis, individual P2 values
< 0.0031 were considered significant (using a nominal 0.05 level for
significance and a rigorous Bonferroni approach) (24). However, P
values < 0.01 are highly suggestive of an association.

Survival durations were computed from the date the patient was
diagnosed with neuroblastoma until the date of death or last follow-up.
Probabilities of survival as a function of time were determined by the
Kaplan-Meier method (25). The Mantel-Haenszel procedure (26) was
used to evaluate the usefulness of each prognostic factor (i.e., age, stage,
makers of differentiation, Shimada grade, and serum ferritin levels) in
predicting patient survival. This procedure was also used to determine
the significance of the difference between a pair of Kaplan-Meier
curves. The Cox proportional hazards modeling technique (27) was
used to identify which factors, when simultaneously evaluated, may
have statistical significance in predicting patient survival. The relative
risk of dying during any short interval of time for each variable in-
cluded in a Cox model was determined as exp (βh), where βh is the
resulting model parameter estimate and exp(a) = 2.71828^a (27).

Results
We have previously presented a model of human adrenal med-
illary histogenesis that incorporates the chromaffin, gangli-
onic, and sustentacular lineages known to constitute the paren-
chymal cells of the adult adrenal medulla (Fig. 1) (17, 23).
CGA, TH, and B2M are markers of the chromaffin lineage; S-100 is a marker of sustentacular cells; and HNK-1 is a marker of fetal adrenal medullary ganglion cells. Within the chroma-
fin lineage, TH and CGA are expressed early during the first
trimester of development, whereas B2M is not expressed until
just before birth. HNK-1 marks a fetal ganglionic lineage that
appears to undergo developmental cell death at ~ 24 wk of
gestation (23). This antibody also marks a small percentage of
chromaffin adrenal medullary cells beginning ~ 4-8 mo after
birth, but does not react with most chromaffin cells until after 5
yr of age (23). In neuroblastoma tumor specimens, HNK-1
immunoreactivity is almost uniformly associated with gangli-
onic differentiation, based on the lack of chromaffin marker
expression in these cell types, as well as the localization of
HNK-1 staining to tumor-derived neurophil (our unpublished
data).

To determine whether neuroblastoma tumor cells corre-
spond to fetal adrenal medullary progenitor cells that have ma-
tured along chromaffin, ganglionic, or sustentacular lineages,
we examined the expression of adrenal medullary lineage-re-
lated markers in 81 tumor samples obtained from surgical spec-
imens of untreated neuroblastoma patients at the Children’s
Hospital of Philadelphia between 1972 and 1987. The clinical
characteristics of this patient population closely reflect those
previously reported for patients with neuroblastoma (Tables I
and III) (6, 9). The proportion of patients with advanced stage
tumors in this cadre is decreased, since the diagnosis of stage IV
neuroblastoma can often be made by bone marrow examina-
tion alone, and primary tumor specimens were therefore not
available for all such patients.

We observed in these specimens that the expression of de-
velopmentally regulated markers associated with either the chro-
malign or sustentacular lineages were unrelated to most previously described prognostic indicators of patient survival, including the age of the patient at diagnosis, serum ferritin level, and histopathologic classification of the tumor (Table I). In contrast, evidence of ganglionic differentiation or absence of all marker expression, designated as a null category, were associated with multiple prognostic indicators. Of particular interest is the association between stage IVS disease and the ganglionic lineage ($P_2 = 0.0055$) and the correlation between the null category and an unfavorable Shimada grading ($P_2 = 0.0018$). This later result was anticipated, since all tumors in the null group were histologically undifferentiated and, therefore, would likely score in the unfavorable category of the Shimada classification.

While all tumors in the null category were histologically undifferentiated, not all undifferentiated tumors were null; some expressed markers of neural crest differentiation. Presented in Fig. 2 are tumor specimens from two undifferentiated neuroblastomas evaluated for TH expression (A and C) and HNK-1 immunoreactivity (B and D). These small, round, blue cell tumors are histologically indistinguishable from each other and from other null category tumors. The tumor in Fig. 2, C and D has differentiated along a chromaffin pathway, as indicated by brown immunoperoxidase staining of tumor cells for TH. In contrast, the tumor in Fig. 2, A and B has differentiated along a ganglionic lineage, as indicated by HNK-1 immunoreactivity without TH expression. These findings demonstrate that histologically indistinguishable neuroblastoma tumors can be recognized to have evidence of differentiation along different lineages and indicate that tumor cells may differentiate along distinct lineages in the absence of morphologic evidence of tumor cell maturation.

Although the tumor specimens presented in Fig. 2 are composed of cells that have differentiated along a single lineage, other tumor specimens were composed of groups of cells that expressed markers from different adrenal medullary lineages. Populations of chromaffin and nonchromaffin tumor cells tended to be localized to different areas of the tumor specimen (data not shown). Of the 66 tumors that expressed lineage-related markers, 30% expressed markers from a single lineage, whereas 70% expressed markers from multiple lineages (data not shown). Since neuroblastoma is thought to be a clonal tumor (29), this finding suggests that some neuroblastoma tumor cells retain the plasticity to differentiate along multiple developmental pathways.

Most neuroblastoma cell lines appear to correspond to adrenal medullary chromaffin progenitor cells arrested at one of several different stages of maturation (17). We therefore evaluated the expression of several developmentally regulated chromaffin markers in neuroblastoma tumor specimens. Presented in Table II are the numbers of tumor specimens that expressed either markers of early chromaffin adrenal medullary maturation, TH and/or CGA, or a marker of mature embryonal chromaffin adrenal medullary cells, B2M. All B2M positive tumor specimens also expressed the early chromaffin markers. Significantly, there were no examples of tumor specimens that expressed B2M without coexpression of early chromaffin markers ($P_2 < 10^{-5}$), the single combination that would have been inconsistent with the pattern of markers expressed during normal development. This finding indicates that chromaffin markers are not expressed in a random fashion. Rather, the observed pattern of gene expression closely mimics that found during embryonal development of chromaffin adrenal medullary cells.

These data indicate that neuroblastoma tumor cells may correspond to the arrested differentiation of adrenal medullary precursors at different points along maturational pathways. We reasoned that the maturational stage or the lineage to which the tumor corresponds may correlate with distinct tumor cell properties, including various features of the tumor that are of clinical significance. We evaluated this possibility by examining the prognostic value of lineage-related marker expression, as well as other previously described prognostic indicators, including age at diagnosis, stage, serum ferritin level, and the Shimada classification. The N-myc copy number and DNA ploidy analysis were not available for most of these patients. As summarized in Table III, we found that evidence of differentiation along a fetal ganglionic lineage, as marked by HNK-1 immunoreactivity, was a significant prognostic variable in this univariate analysis, comparable to these other prognostic indicators. The expression of other developmentally regulated markers was not of prognostic significance.

To evaluate if HNK-1 was an independent prognostic indicator of patient survival, we performed a Cox proportional hazards analysis including patient age at diagnosis, stage, serum ferritin level, and lineage-related marker expression in the models. As the Shimada classification incorporates the age of the patient at diagnosis in its formulation, it could not be considered independent of age, and therefore was not included in this analysis. The most predictive model we could identify (model chi square value of 44.25 with three degrees of freedom) included only three variables: HNK-1 immunoreactivity, with a relative risk of 6.42 for those patients having HNK-1 negative tumors ($P_2 = 0.0001$); age at diagnosis, with a relative

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**Table I. Correlation between the Expression of Lineage-related Markers and Prognostic Indicators of Neuroblastoma Patient Survival**

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Tumors analyzed</th>
<th>Chromaffin</th>
<th>Sustentacular</th>
<th>Ganglionic</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 yr</td>
<td>36</td>
<td>24</td>
<td>9</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>≥ 1 yr</td>
<td>45</td>
<td>28</td>
<td>22</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II</td>
<td>32</td>
<td>22</td>
<td>15</td>
<td>20*</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>24</td>
<td>16</td>
<td>9</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>IVS</td>
<td>9</td>
<td>6</td>
<td>1</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>61</td>
<td>42</td>
<td>25</td>
<td>39</td>
<td>7*</td>
</tr>
<tr>
<td>High</td>
<td>19</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Shimada</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>43</td>
<td>31</td>
<td>22</td>
<td>31*</td>
<td>3*</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>34</td>
<td>17</td>
<td>8</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

* The stage reported is the Evans staging system (28). Patients with serum ferritin levels > 150 ng/ml were considered elevated (9). Tumors were classified according to the Shimada grading as previously reported (11). \* Groupings by stage are as follows: I + II, III + IV, IVS ($P_2 = 0.005$); I + II + III + IV, IVS ($P_2 = 0.0055$). \* $P_0.0031 \leq P_2 < 0.01$. \* $P_2 < 0.0031$ (significant, see text).
Figure 2. Histologically indistinguishable neuroblastoma tumors have different patterns of lineage-related gene expression. Two neuroblastoma tumor specimens with undifferentiated histopathologic features (tumors 1 and 2) were evaluated by immunoperoxidase staining for TH expression (A and C) and HNK-1 (B and D) immunoreactivity. ×190.

Discussion

Although numerous lines of investigation indicate that malignant cells from aggressive solid tumors often display poorly differentiated features (30), there is little evidence suggesting a correspondence between solid tumor cells and the cell types found during histogenesis of tissues in which such tumors arise. The availability of multiple chromaffin-related developmental markers made it possible to critically evaluate the hypothesis that neuroblastoma tumor cells correspond to the arrested differentiation of chromaffin adrenal medullary progenitor cells. As reported in Table II, we found no example of chromaffin-related marker expression in neuroblastoma tumor specimens that deviated from the normal developmental pattern of gene expression. These findings indicate that chromaffin gene expression in neuroblastoma is not a stochastic process. Rather, these data strongly argue that some of the regulatory mechanisms that control chromaffin maturation in normal adrenal

Table II. Neuroblastoma Tumor Cells Correspond to the Arrested Differentiation of Chromaffin Adrenal Medullary Progenitor Cells

<table>
<thead>
<tr>
<th>Chromaffin markers*</th>
<th>Tumors with designated pattern of marker expression†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (TH/CGA)</td>
<td>Late (B2M)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* The markers evaluated included TH, CGA, and B2M. +, detectable immunoreactivity; –, no immunoreactivity was detected. †P2 < 10⁻³ by the chi-square test.
Expression and Survival of THNKL

Median follow did progenitor correspond S-100 that these assay pathways. proved survival tumors (Table 2406 M.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>5-yr survival (%)</th>
<th>$P_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 Year</td>
<td>36</td>
<td>89</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>≥ 1 Year</td>
<td>45</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I, II</td>
<td>32</td>
<td>81</td>
<td>0.0026$^+$</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>56</td>
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</tr>
<tr>
<td>IV</td>
<td>24</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>IVS</td>
<td>9</td>
<td>89$^+$</td>
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<td>Ferritin</td>
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<td></td>
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<tr>
<td>Low</td>
<td>61</td>
<td>77</td>
<td>&lt; 0.0001</td>
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<tr>
<td>High</td>
<td>19</td>
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<tr>
<td>Shimada</td>
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<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>43</td>
<td>91</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>34</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>TH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>47</td>
<td>68</td>
<td>0.48</td>
</tr>
<tr>
<td>Negative</td>
<td>34</td>
<td>59</td>
<td></td>
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<tr>
<td>CGA</td>
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<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18</td>
<td>66</td>
<td>0.93</td>
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<tr>
<td>Negative</td>
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<td>63</td>
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<td>B2M</td>
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<tr>
<td>Positive</td>
<td>26</td>
<td>57</td>
<td>0.94</td>
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<td>55</td>
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<td>31</td>
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<tr>
<td>Negative</td>
<td>50</td>
<td>60</td>
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<tr>
<td>HNK-1</td>
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<tr>
<td>Positive</td>
<td>46</td>
<td>83</td>
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<tr>
<td>Negative</td>
<td>35</td>
<td>34</td>
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</table>

* Median follow up of survivors is 86 mo. $^+$ Stages I + II compared to stages III and IV. $^+$ The sample size for this group is small; 95% confidence interval is 57–98%.

Embryonal neuroblasts are operative in neuroblastoma tumor cells.

The lack of multiple sustentacular and ganglionic markers did not allow us to determine if neuroblastoma tumor cells also correspond to the arrested maturation of adrenal medullary progenitor cells that have differentiated along these pathways. Nevertheless, S-100 and HNK-1 immunoreactivity provides an assay to determine if a tumor cell has matured along nonchromaffin pathways. As presented in Table I, many tumor specimens expressed nonchromaffin markers, suggesting that these developmental pathways of neural crest origin, as well as the chromaffin lineage, are important in the biology of this tumor.

This developmental analysis of neuroblastoma has identified a differentiation pathway associated with tumors that are responsive to antineoplastic therapies and/or lack aggressive potential. Patients with tumors that had immunohistochemical evidence of ganglionic differentiation had significantly improved survival compared to patients having HNK-1 negative tumors (Table III). As illustrated in Fig. 3, both age at diagnosis and HNK-1 immunoreactivity can independently distinguish subsets of good prognosis neuroblastoma patients from larger groups of high risk patients defined by use of either variable alone.

Many neuroblastoma tumors retain the plasticity to mature along multiple adrenal medullary lineages. Of the 66 tumors that expressed lineage-related markers, 70% contained different populations of cells expressing markers from two or more adrenal medullary lineages. Patients with HNK-1 positive tumors had a good prognosis irrespective of the percentage of tumor cells expressing this marker or whether other tumor cells in the specimen were marker negative or had differentiated along chromaffin or sustentacular pathways (data not shown). These data suggest that the biological significance of HNK-1 immunoreactivity in predicting patient survival is the plasticity of tumor cells to mature along a fetal ganglionic lineage. This tumor plasticity may be mediated through intrinsic pathways characteristic of neural crest progenitor cells during embryogenesis (31, 32), and may result from the presence of extrinsic modulators of neural crest cell differentiation (33, 34). Several agents that can modulate adrenal medullary differentiation, including glucocorticoids (35–37), fibroblast growth factor (38, 39), and nerve growth factor (35–37) have been identified in model systems of neural crest maturation. In addition, the presence of local growth factors normally produced by the adrenal cortex, such as IGF-II, may also influence the proliferation and differentiation of neuroblastoma tumor cells (40).

The association between HNK-1 immunoreactivity and improved patient survival suggests that therapeutic strategies to differentiate neuroblastoma tumors along a ganglionic lineage may be successful in modulating the therapeutic responsiveness of these tumors. In vitro studies using neuroblastoma cell lines indicate that some biological response modifiers, such as retinoic acid and dexamethasone, can modulate chromaffin-related marker expression (41–42) (data not shown). Related studies may identify subsets of neuroblastoma patients whose tumor cells retain the plasticity to mature along a ganglionic pathway, suggesting a novel approach for the use of biological response modifiers to modulate tumor cell biology and render currently incurable patients sensitive to available antineoplastic therapies.

This developmental analysis of neuroblastoma also suggests a mechanism for the spontaneous regression of stage IVS tumors. In previous studies, we have reported that the number of ganglion progenitor cells in fetal adrenal glands dramatically falls after 24 wk of gestation (23). Since many fetal ganglion cells have evidence of nuclear fragmentation at this developmental stage (data not shown), we believe that most fetal adrenal ganglion cells undergo developmental cell death. As presented in Table I, our analysis revealed that all nine stage IVS tumor specimens were HNK-1 positive compared to 37 of 72 non-stage IVS tumors, an association with near statistical significance in view of the numerous comparisons being made in this retrospective analysis ($P_2 = 0.0055$). These data raise the possibility that the spontaneous remission of stage IVS disease may be related to mechanisms underlying the programmed cellular death of fetal adrenal medullary ganglion cells. The potential to induce tumor cells to undergo cell death might well be pursued in neuroblastoma.

The composition of our patient population by age and stage is essentially identical to other reports (6, 43), except that stage IV patients account for 30% of our population compared to...
APATIENTS

include

excluded solely

mors, mental marker previously tabulated patients did not react to immunoreactivity medullary progenitors lineage same to epithelial and bladder may lial progenitor cells recent may arrest. In summary, these studies demonstrate that neuroblastoma tumors consist of cells that correspond to embryonal adrenal medullary progenitors arrested at recognizable stages in their maturation; that evidence of maturation along a fetal ganglionic lineage appears to have major importance in predicting patient survival; and that biologically distinct subgroups of the same tumor type that have differing responses to antineoplastic therapy and/or vary in their malignant potential can be recognized by an evaluation of lineage-related gene expression. It is possible that other pediatric solid tumors may also correspond to the arrested differentiation of tissue progenitor cells, and recent reports suggest that rhabdomyosarcoma and Wilms' tumor may fit this paradigm (44, 45). Although it is currently unknown whether common adult tumors, such as colon, lung, and bladder cancers, correspond to the arrested maturation of epithelial cells at different stages of cell renewal and differentiation, it is conceivable that the complex biology of these tumor types may be related to distinct biological properties of epithelial progenitor cells at different stages of tissue maturation.

Acknowledgments

We gratefully acknowledge the generous support of this research by the Preus Foundation.

References


Figure 3. Survival of neuroblastoma patients by age at diagnosis, stage, and HNK-1 immunoreactivity. (A) Kaplan-Meier survival curves of patients > 1 year of age (n = 45) by presence or absence of HNK-1 immunoreactivity (P < 10−6). (B) Kaplan-Meier survival curves of patients having HNK-1 negative tumors (n = 35) by age at diagnosis (P2 = 0.0008). (C) shows the survival of advanced stage neuroblastoma patients (stages III and IV, n = 40) by presence or absence of HNK-1 immunoreactivity (P2 = 0.0008). (D) Kaplan-Meier survival curves of patients having HNK-1 negative tumors (n = 35) by stage (P2 = 0.026).

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