Focus on embryonal malignancies

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Introduction, epidemiology, and natural history

The majority of pediatric cancers likely reflect the inherent risks associated with the complex process of normal development rather than aberrant responses to external insults. Epidemiologic data has not identified any consistent environmental factors, including parental exposures to carcinogens, which have been consistently linked to tumor predilection (Ross and Swensen, 2000). It therefore seems likely that many pediatric tumors may be an infrequent and unfortunate consequence of normal growth and development gone awry (Figure 1). Nowhere is this concept more evident than in pediatric embryonal malignancies—solid tumors arising from cellular populations that have not completed the process of terminal differentiation during fetal and/or postnatal development. While many pediatric cancers are thought to arise from blastemal cells, this review will focus on neuroblastoma, retinoblastoma, nephroblastoma (Wilms' tumor), and hepatoblastoma as examples of the unique biological and clinical features inherent to the solid malignant neoplasms of childhood.

While the four major embryonal malignancies account for only slightly less than 20% of cancer observed in children less than 15 years of age (Ries et al., 1999), they are by far the most common neoplasms detected during very early childhood. The median age of diagnosis for the embryonal cancers ranges from approximately 1.5–3 years of age (Table 1), and the majority of cases are diagnosed before the 5th birthday. Congenital cases are relatively frequent, especially for retinoblastoma and neuroblastoma, strongly suggesting that disruption of normal developmental processes are involved in tumor initiation.

The natural history of neuroblastoma highlights many of the unique features and conundrums of pediatric embryonal malignancies. On the one hand, neuroblastoma shows the highest spontaneous tumor regression rate of all human malignancies. There are patients in the first year of life with a special pattern of disseminated disease (Stage 4S) that can be readily identified and treated with observation alone despite rapid initial tumor progression. On the other hand, approximately 50% of patients are older children with disseminated disease and show relentless tumor progression. These tumors are frequently refractory to conventional dose-intensive therapies (despite initial responses), and overall survival remains less than 40% (Matthay et al., 1999). Although 90% of cases are diagnosed before age 5, neuroblastoma does occur in older children, and very rarely adults, where it typically

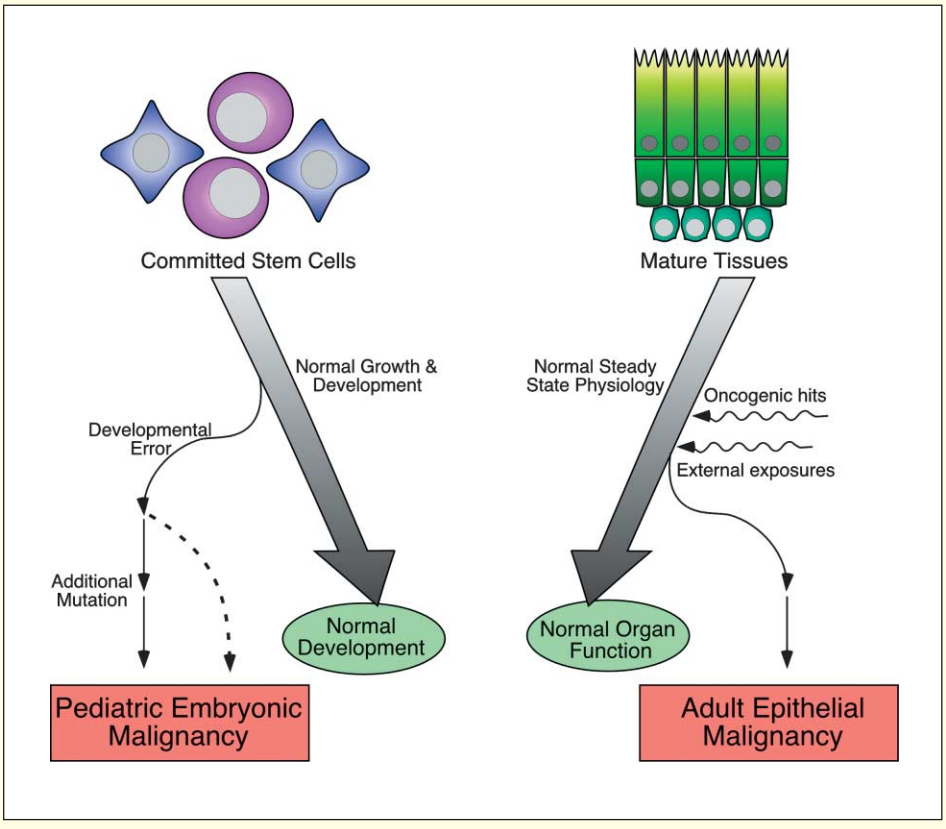

Figure 1. Putative oncogenic mechanisms of pediatric embryonal and adult epithelial tumors

Embryonal tumors presumably originate from stem cell populations in organs and structures that are undergoing large-scale cell division and differentiation as part of normal growth and developmental (broad arrow). Infrequent errors occur that could involve alterations in chromatin structure or discrete genomic mutations (thin arrows). In some cases, these errors directly confer a growth advantage to cells (dashed arrow) or predispose cells to accumulate additional mutations that eventually lead to tumor formation. Adult epithelial tumors arise from stem cell populations that normally function in mature organs as a replacement source for those cells constantly lost as a part normal steady state physiology. Exposure to external stimuli and/or de novo genomic mutation results in expansion of this stem cell population. This increases the risk of incurring additional mutations and a resultant malignant clonal outgrowth. Germline alterations in cancer predisposition genes provide a shortcut to malignancy in both cases.

Table 1. Clinical and genetic features of embryonal malignancies

Tumor	Incidence[1]	Median age diagnosis (months)	Associated conditions	Overall survival[3]	Predisposition genes/loci[4]
Neuroblastoma	9.1	17	Hirschsprung disease Central hypoventilation	64%	*HNB1* (16p12-13) 1p36.3 11q14-23
Wilms'	8.1	39	WAGR[2] Denys-Drash Hemihypertrophy Beckwith-Wiedemann	92%	*WT1* (11q13) *FWT1* (17q12-21) *FWT2* (19q13)
Retinoblastoma	4.0	24	13q deletion syndrome	94%	*RB1* (13q14)
Hepatoblastoma	1.3	16	FAP Beckwith-Wiedemann	59%	*APC* (5q22)

[1]Average age-adjusted annual incidence rate per million children < 15 years of age (Ries et al., 1999).
[2]WAGR: Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation syndrome.
[3]Five-year relative survival rates estimated between 1985–1994 (Ries et al., 1999).
[4]Constitutional heterozygous alterations at these loci predispose to neoplasm. *HNB1*, *FWT1*, and *FWT2* are postulated genes at regions identified by genetic linkage analysis. 1p36.3 and 11q14-23 are sites of constitutional deletions identified in patients with multifocal neuroblastoma.

follows a more indolent course but ultimately is fatal (Franks et al., 1997). These clinical data suggest that there is a narrow window of opportunity to accumulate the required genomic alterations in susceptible committed, but not fully differentiated, blastemal cells. The fact that forced *MYCN* expression to the murine peripheral neural crest causes neuroblastomas at high penetrance, but only within the first several months of life, reinforces the concept of a vulnerable time period prior to terminal differentiation (Weiss et al., 1997).

Genetics

Genetic predisposition

Retinoblastoma provides the paradigm for heritable predisposition to cancer segregating as a dominant trait. Knudson's mathematical prediction that retinoblastomas arise due to two separate mutational events (Knudson, 1971) was validated by the discovery of germline heterozygous mutations in *RB1* with somatically acquired inactivation of the other allele. Although some exceptions do occur, *RB1* germline mutations are typically highly penetrant, suggesting that even a single genomic hit is sufficient to initiate the oncogenic process. However, additional mutations are usually necessary for tumor formation. Essentially all retinoblastomas show multiple other karyotypic alterations, including gain of 1q and 6p, loss of 16q, and high level amplification of the *MYCN* protooncogene (Chen et al., 2001). Thus, even the most straightforward pediatric embryonal cancer shows genomic alterations that suggest that establishment of a fully malignant phenotype requires multiple somatically acquired alterations. It remains a complete mystery as to why germline mutations in *RB1* result in strict tissue specificity (retina and bone) for neoplasia, yet acquired inactivation of *RB1* is an essential step toward the malignant phenotype in multiple human cancers (Hanahan and Weinberg, 2000).

Heritable susceptibilities in the other embryonal cancers appear more complex. The *WT1* tumor suppressor gene is mutated in about 10% of sporadic Wilms' tumors, but germline mutations in this gene are rare. There are at least two other predisposition loci at 17q12-21 (*FWT1*) and 19q13 (*FWT2*) and probably others (Huff et al., 1997). Familial neuroblastoma is also likely to be genetically heterogeneous, but a major predisposition gene has been mapped to 16p12-13 (Maris et al., 2002). Hepatoblastomas have only very rarely been reported in siblings, but heritable predisposition does occur in patients with germline mutations of the adenomatous polyposis coli (APC) gene (Giardiello et al., 1996). Constitutional chromosomal abnormalities and associated congenital anomalies are rare in patients with embryonal cancers, but these unique patients have directly contributed to the discoveries of *RB1* and *WT1* and may be important for gene identification in other pediatric cancers (Table 1).

Somatically acquired genomic alterations

Despite the generally held notion that embryonal cancers are simpler from a genetic standpoint than adult neoplasms, these cancers show multiple chromosomal rearrangements, suggesting a complicated series of acquired alterations during malignant evolution. This is best exemplified in neuroblastoma, where 20%–25% of cases harbor multiple copies of the *MYCN* gene with concomitant overexpression of the mRNA and protein. This mutation has a profound effect on tumor clinical behavior. In addition, metastatic neuroblastomas are generally diploid (or near tetraploid) and show structural rearrangements, including loss of heterozygosity (LOH) at 1p36, 3p14-25, 11q14-23 and 14q32, as well as unbalanced gain of 17q23-25 (Maris and Brodeur, 2001). This suggests that there can be additional biologically relevant genetic hits in neuroblastoma, though the involved genes remain to be elucidated.

Secondary genetic hits in other embryonal tumors are less well defined. Activating mutations in *CTTNB1* (β-catenin) are frequent in both hepatoblastomas and Wilms' tumors (Koch et al., 1999; Koesters et al., 1999). In addition, there are multiple regions of the genome that are commonly and recurrently deleted in each of the embryonal cancers, and epigenetic events may play an important role in functional inactivation of putative suppressor genes at these loci. *TP53* mutations, which are a common finding in many adult cancers, are not common initially in embryonic tumors, but acquired mutations do occur under the selective pressure of chemotherapy and may contribute to treatment failures (Keshelava et al., 2001).

Conventional therapeutics

Like all human malignancies, the major variable affecting treatment planning is anatomic extent of disease. Age at diagnosis is another important variable, both in terms of age being a surrogate marker for disease aggressiveness and likelihood for morbidity to cytotoxic therapy. There has been a recent trend toward combining these clinical features with tumor-specific biological

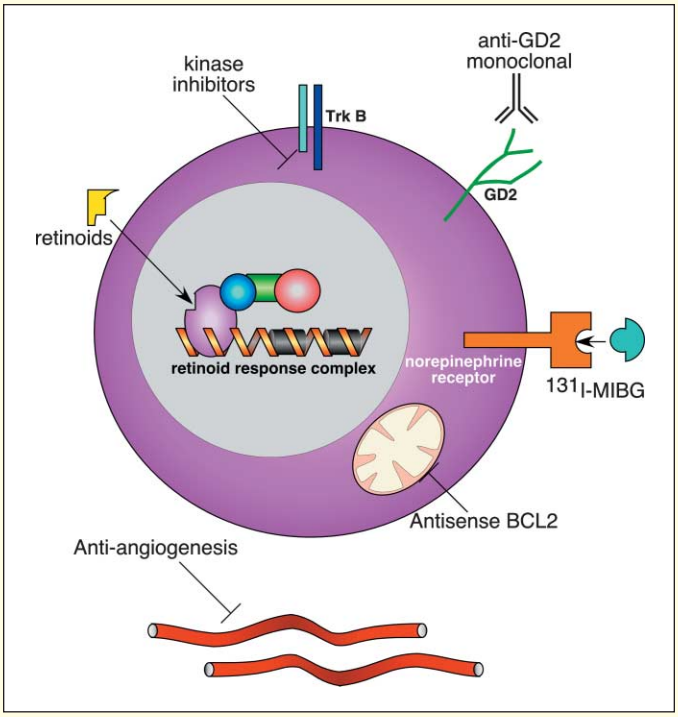

Figure 2. Novel therapeutic strategies in neuroblastoma

A subset of the most promising new therapeutic approaches currently in clinical trials or in late preclinical development.

prognostic markers in order to stratify patients into risk groups. This is perhaps best formulated in neuroblastoma, where the tumor biological features of *MYCN* amplification and DNA index (ploidy) are independently prognostic for disease outcome and are used clinically to assign treatment intensity. Other somatically acquired genomic alterations, such as 1p36 LOH and/or unbalanced gain of 17q23-25, might add further precision to the current risk stratification scheme. Future Wilms' tumor cooperative group trials will stratify therapy based on 1p and 16q LOH status.

The embryonal cancers can be exquisitely chemo- and radiation-therapy responsive. Overall survival rates continue to improve, and for retinoblastoma and Wilms' tumor have approached the point where cure is the expectation (Table 1). Even the most aggressive neuroblastomas with *MYCN* amplification typically show good initial responses to cytotoxic therapy. The vast majority of patients with localized neuroblastoma can be cured with surgery alone, and most hepatoblastomas and "intermediate risk" neuroblastomas can be cured with relatively modest neoadjuvant chemotherapy. For these cancers, current emphasis is on therapy reduction in order to avoid late morbidity. However, survival rates for metastatic neuroblastoma remain suboptimal (3-year EFS 30%),despite escalating chemotherapy intensity to the point of using myeloablative chemotherapy with autologous, purged bone marrow rescue (Matthay et al., 1999).

Novel treatment approaches

The distinctive oncogenic mechanisms present in embryonal malignancies suggest that their inherent physiologic state and protein expression profile are quite different than host somatic tissues. Such proteins and pathways could provide attractive targets for novel treatment strategies (Figure 2). For example, recognition of the adrenergic phenotype of neuroblastomas has led to the development of metaiodobenzylguanidine (MIBG) as both a diagnostic and therapeutic agent. Targeted radiotherapy with ^{131}I labeled MIBG has shown extraordinary responses in highly refractory disease, even in tumors that have progressed following myeloablative chemotherapy, with minimal non-hematopoietic toxicity (Matthay et al., 1998). The observation that TrkB is preferentially expressed in metastatic neuroblastoma suggests that the neurotrophin signaling pathway might also provide a relatively specific target. Targeted inhibition of Trk receptors tyrosine kinase activity showed a significant growth inhibitory effect on several human-derived neuroblastoma xenografts and efficacy was related to TrkB expression level (Evans et al., 1999).

Intervention through transcriptional modulation is another therapeutic venue in embryonal malignancies. *MYCN* provides an obvious target for the subset of neuroblastomas (and retinoblastomas) with genomic amplification, but attempts to exploit this with antisense or other targeted approaches have been fraught with difficulties. Interfering with the mechanisms allowing for escape from apoptosis is appealing, especially in *MYCN* amplified cells where the oncogene itself provides a powerful apoptotic stimuli that must be circumvented. Antisense oligonucleotides to *BCL2* are currently in clinical trials, and demethylating agents (e.g., decitabine) are being considered following the observation that *MYCN* amplification is often associated with loss of caspase 8 expression due to promoter site hypermethylation (Teitz et al., 2000). Based on their ability to induce differentiation in tissue culture systems, retinoids have had a positive clinical impact in neuroblastoma. Encouraging results from a randomized trial of 13-*cis*-retinoic acid (Matthay et al., 1999) has fueled efforts to define retinoic acid isomers that promote maximum tumor differentiation (or kill) without excess toxicity (Reynolds, 2000).

Novel immunotherapeutic strategies targeting neuroblastoma cell surface structures have received significant attention in neuroblastoma. The disialoganglioside GD2 is highly expressed on the cell surface of virtually all neuroblastoma cells, while being present on only a restricted range of normal tissues, including peripheral nerves. Monoclonal antibodies against GD2 show clinical activity, and antibody-dependent cytotoxicity can be enhanced with concomitant administration of cytokines (Ozkaynak et al., 2000). An anti-GD2 monoclonal antibody combined in alternating cycles with GM-CSF or IL2 is currently being studied in a randomized trial on the backbone of 13-*cis*-retinoic acid for neuroblastoma patients in first complete or very good partial response. A variety of other immunotherapeutic approaches are also currently under investigation, including vaccines (cytokine transfected autologous tumor cells, dendritic cell, anti-idiotype), anti-GD2-cytokine fusion antibodies, and ex vivo T cell augmentation.

Finally, efforts to modulate the extracellular environment are being pursued. Antiangiogenic strategies hold promise for pediatric solid cancers, especially neuroblastoma, where high-risk disease features are correlated with primary tumor vascularity (Meitar et al., 1996). It seems likely that process of new vessel formation in embryonal malignancies may involve both vasculogenesis (de novo assembly of endothelial precursor cells) and angiogenesis (recruitment of angiogenic sprouts from the existing vasculature), which may predict for differential sensitivity to antiangiogenic strategies being explored in adult neoplasms. Antiangiogenic strategies have shown promise in preclinical

models of neuroblastoma and Wilms' tumor (Kim et al., 2001). Current preclinical studies are focused on combination strategies with conventional or low-dose cytotoxic chemotherapy (Klement et al., 2000). The unique problem of defining exactly how to use neovascular inhibition strategies in growing children has not yet been addressed, as protracted chronic inhibition schedules to sustain tumor dormancy are unlikely to be useful in the pediatric population.

Challenges for the future

Perhaps because they reflect corrupted normal developmental pathways, embryonal tumors have provided a fruitful area of basic science research, including discoveries of many of the paradigms of modern cancer genetics. A striking observation is that in spite of shared basic oncogenic mechanisms with adult cancers, pediatric embryonic tumors are virtually never seen after the first decade of life. This could reflect biological differences in respective stem cell populations that are targeted in adult versus pediatric malignancies. The specificity of genetic mutation is also likely to play a key role. Finally, the differences in cellular growth environments between developing pediatric and mature adult host tissues may favor certain types of cancer over others. Future studies in pediatric embryonic tumors will address these issues and provide an invaluable opportunity toward better understanding of both normal and abnormal human growth and development.

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