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Genetic Discoveries and Treatment Advances in Neuroblastoma

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Abstract

Purpose of review—Major advances in our understanding of the genetic basis of neuroblastoma and the role somatic alterations play in driving tumor growth have led to improvements in risk-stratified therapy and have provided the rationale for targeted therapies. In this review, we highlight current risk-based treatment approaches and discuss the opportunities and challenges of translating recent genomic discoveries into the clinic.

Recent Findings—Significant progress in the treatment of neuroblastoma has been realized using risk-based treatment strategies. Outcome has improved for all patients, including those classified as high-risk, although survival remains poor for this cohort. Integration of whole-genome DNA copy number and comprehensive molecular profiles into neuroblastoma classification systems will allow more precise prognostication and refined treatment assignment. Promising treatments that include targeted systemic radiotherapy, pathway-targeted small molecules, and therapy targeted at cell surface molecules are being evaluated in clinical trials, and recent genomic discoveries in relapsed tumor samples have led to the identification of new actionable mutations.

Summary—The integration of refined treatment stratification based on whole-genome profiles with therapeutics that target the molecular drivers of malignant behavior in neuroblastoma has the potential to dramatically improve survival with decreased toxicity.

Keywords

neuroblastoma; risk classification; molecular profile; targeted therapy; immunotherapy

Introduction

Neuroblastoma is noteworthy for its broad range of clinical behavior and variable response to treatment.¹ Tailored treatment approaches, based on a combination of clinical, histologic, and genetic prognostic factors have led to significant improvements in survival. However,

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Conflicts of Interest

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outcome remains poor for patients classified as high-risk, with <50% achieving long-term survival.¹ High-risk survivors are at risk for significant treatment-related adverse events including subsequent malignancies,² emphasizing the critical need for more effective, less toxic treatments. In this review, we discuss recent progress in our understanding of the molecular underpinnings of the etiology and pathobiology of neuroblastoma. We also highlight “omic” profiles that offer more precise prognostic information than established classifiers, and new genomic discoveries that provide the rationale for evaluating targeted therapeutics.

Genetic Epidemiology of Neuroblastoma

Insight regarding the genetic etiology of neuroblastoma was first provided through linkage studies conducted in rare familial neuroblastoma cases. Inherited mutations in *PHOX2B* and *ALK* have been identified in these pedigrees, although disease penetrance is incomplete.³ In sporadic cases, these familial predisposition genes do not play a causative role in the vast majority of patients. Rather, genome-wide association studies (GWAS) have led to the identification of common genetic variants that contribute to disease susceptibility. Interestingly, many of the susceptibility variants are associated with tumor phenotype. *DUSP12*, *HSD17B12* and *DDX4-IL31RA* are associated with susceptibility to low-risk neuroblastoma, whereas single nucleotide polymorphisms (SNP) within or upstream of *CASC15* and *CASC14*, *BARD1*, *LMO1*, *HACE1* and *LIN28B* as well as a common copy-number variation (CNV) at 1q21 within *NBPF23* have been shown to be highly enriched in the cohort of patients with clinically aggressive, high-risk disease.⁴ Further, two rare germline *TP53* variants have also been associated with neuroblastoma.⁵ Many of these SNPs influence gene expression, and functional studies have shown that *LMO1*, *BARD1*, and *LIN28* promote neuroblastoma growth.^{6–8} In contrast, tumor suppressor activity is associated with a short isoform of the long noncoding RNA, *CASC15* (*CASC15-S*).⁹

Common genetic variants also contribute to the higher prevalence of high-risk disease and worse outcome observed in African American children with neuroblastoma compared to Caucasians.¹⁰ Ongoing deep sequencing studies in patients with mixed lineage will lead to a deeper understanding of the genetic factors contributing to the etiology of this cancer and the disparities in survival.

Somatic Mutations

Amplification of *MYCN* is the most common somatic alteration in neuroblastoma. Although this oncogene was first discovered more than 30 years ago, *MYCN* status remains a powerful biomarker for treatment stratification today. Next-generation sequencing has revealed a relative paucity of other genomic aberrations in neuroblastoma tumors.^{11,12} *ALK* amplification and activating mutations of the *ALK* tyrosine kinase receptor occur in approximately 14% of all sporadic neuroblastoma, with the most frequent mutations at F1174 and R1275.¹³ *ATRX* mutations have been identified in <10% of cases, although nearly half of adolescents and young adults with neuroblastoma bear somatic mutations in this gene.¹⁴ Other recurrent mutations that occur at low frequency include heterozygous missense *PTPN11* mutations¹² and truncating mutations of the *TIAM1* gene.¹¹ Rare

mutations in *MYCN* have been detected in *MYCN*-nonamplified tumors, and mutations in *ARID1A* and *ARID1B* have also been reported.¹⁵ Although few actionable mutations are detected at the time of diagnosis, recent studies indicate that the frequency of RAS-MAPK pathway mutations is higher in relapsed neuroblastoma, suggesting that treatments targeting this pathway may be effective in relapsed or refractory disease.¹⁶

Risk Classification

Children with neuroblastoma have received risk-based treatment for decades. To create a uniform, international pre-treatment classification algorithm, the International Neuroblastoma Risk Group (INRG) Task Force analyzed data collected on more than 8,800 patients and 36 prognostic factors.¹⁷ Although implementation of this system in the Children's Oncology Group (COG) and International Society of Paediatric Oncology Europe Neuroblastoma (SIOPEN)¹ will facilitate comparison of risk-based clinical trials, the limitations of this algorithm are well recognized. Clinical and histologic risk criteria are merely surrogates for underlying tumor biology. Further, the individual genomic prognostic markers (*MYCN* and 11q status) in the INRG system were identified more than 30 years ago, before comprehensive approaches for whole genome analysis were developed. The next generation INRG Classification System is expected to be based on more current and perhaps more therapeutically applicable whole-genome and molecular biomarkers. (Table 1)

Genomic DNA Profiling

Whole-genome DNA copy number analyses have demonstrated that the overall genomic pattern adds significant prognostic information to conventional risk algorithms.¹⁸ Whole chromosome duplications without segmental aberrations are predictive of a favorable outcome, while inferior survival is observed in patients with tumors with segmental aberrations. The number of chromosome breakpoints is also prognostic of outcome; the presence of >7 segmental aberrations is associated with clinically aggressive disease.¹⁹

Expression Signatures

Microarray-based technologies have been used to analyze the neuroblastoma transcriptome, and a number of RNA-based signatures have been shown to have predictive power independent of conventional prognostic variables (reviewed in²⁰). Biology-driven approaches have been used to generate a 62-hypoxia gene prognostic signature,²¹ and a classifier that includes genes detected in tumor-associated inflammatory cells²². Other studies have demonstrated the prognostic value of expression profiling of miRNA, other noncoding RNAs, and epigenetic modifiers.^{20,23} Despite the promise of these studies, molecular signatures have not been widely integrated into risk classification systems, in part due to the requirement for high-quality RNA for microarray-based assays. Digital technologies that accurately quantify gene expression using RNA isolated from formalin-fixed paraffin embedded (FFPE) tissues mitigate this limitation and are more suitable for clinical use.²⁴

Advances in Risk-Stratified Therapy

An overview of the current treatment approach for non-high-risk and high-risk patients is highlighted below. Integration of molecular profiles and genomic markers that are reflective of tumor biology into future classification systems will allow more precise prognostication and refined treatment assignment.

Non-High Risk Disease

Over the past decade, efforts have been made to safely reduce therapy for patients at lower risk of relapse. Subsets of very young children have been shown to have a survival rate 95% without therapy.²⁵ Outcomes for patients with localized disease (INSS 1 and 2; INRG L1) are excellent with surgery alone, provided that the tumors have no unfavorable features.²⁶ Patients with tumors that are unresectable at diagnosis (INRGSS L2 or INSS 3 tumors) have historically been treated with systemic therapy, and the intensity of therapy has been successfully reduced in children with tumors with all favorable features.²⁷ Further reductions in therapy are being evaluated in both European (NCT01728155) and North American trials (NCT02176967).

However, risk assessment is more complex in patients with favorable clinical features and tumors that are found to have unfavorable genomics or histology. Unlike patients with localized resectable tumors with favorable features, patients with diploid tumors or tumors with unfavorable histology have EFS rates of only 61–75% following surgery alone.^{26,28} Similarly, patients with localized unresectable disease whose tumors have segmental chromosome aberrations or unfavorable histology have an increased risk of relapse following moderate intensity systemic therapy.^{29,30} Therapy has not been reduced for infants with Stage M diploid neuroblastoma, as EFS for these patients was just 71% with moderate intensity therapy.²⁷

High Risk Disease

Modern-era therapy for high-risk neuroblastoma has been intensified during the past decade and includes surgery, chemotherapy, radiation, and immunotherapy. This multi-modality treatment is currently delivered to children >18 months of age at diagnosis who have metastatic or unresectable disease with *MYCN* amplification or unfavorable histology, and to children of any age with unresectable or metastatic disease in the presence of *MYCN* amplification. Induction consists of cycles of multi-agent chemotherapy and primary tumor resection.¹ Initial therapy is followed by an augmented consolidation consisting of high dose chemotherapy with autologous stem cell rescue (ASCR) and external beam radiation. While ASCR has been shown to improve EFS in large, randomized trials, OS has not improved in patients enrolled on ASCR-containing regimens.^{31,32} This highlights the need to develop treatments that will improve survival for patients with high-risk disease, and to refine our approach to treatment assignment so that maximally intensive myeloablative therapy is only delivered to those most likely to derive benefit.

Upon recovery from ASCR, external beam radiotherapy is administered. High rates of disease site control have been reported following radiation to the primary tumor bed and

sites of persistent metastatic disease (5 year site-specific control rates of 84% and 74%, respectively).³³ Among patients with relapsed disease, recurrence in bony sites present at diagnosis and treated with radiation was less frequent than recurrence in sites not radiated (16% vs 25%).³⁴ Rates of toxicity to viscerae are low following modern-era radiotherapy, however radiation may be linked to bone changes and second malignancies in survivors of high-risk disease.^{2,35} Identification of markers of radiation responsiveness in neuroblastoma tumors may be helpful in radiation-related decision-making in the future.

Post-consolidation therapy is designed to treat minimal residual disease, and includes administration of dinutuximab, a chimeric antibody directed against the cell surface ganglioside GD2. A randomized COG trial showed improved survival in patients assigned to receive dinutuximab in combination with cytokines (granulocyte-macrophage colony stimulating factor and interleukin-2) and the differentiating agent isotretinoin than in those randomized to isotretinoin alone (2-year EFS 66% versus 46%; 2-year OS 86% versus 75%).³⁶ Strategies to optimize administration of adjuvants that enhance the activity of dinutuximab are being studied in Europe (NCT01704716). Toxicities associated with this post-consolidation regimen are non-trivial; patients frequently experience pain, allergic reactions, blood pressure changes, and fevers. Analysis of the relationship between anti-glycan antibodies and the incidence of allergic reactions is in progress, as are studies to identify Fcγ Receptor, Killer Immunoglobulin-like Receptor (KIR), and KIR ligand polymorphisms associated with the efficacy of GD2-directed therapy.³⁷ In addition, because high levels of the immunosuppressive NKp30c isoform and its ligand B7-H6 have been associated with unfavorable outcomes in children with neuroblastoma,³⁸ studies of the relationship between NKp30 isoform patterns and outcome following immunotherapy are ongoing.

Relapsed Disease

Disease recurrence continues to present a major therapeutic challenge. Approximately 50% of children with high-risk disease will relapse, and 5-year OS among those who relapse is only 8%.³⁹ Several approaches to targeted therapy for relapsed neuroblastoma have been explored, including targeted systemic radiotherapy, pathway-targeted small molecules, and therapy targeted at cell surface molecules.

Targeted radiotherapy

Approximately 90% of neuroblastoma tumors take up radioactive meta-iodobenzylguanidine (¹³¹I-MIBG). This norepinephrine analog has been extensively studied in relapsed and refractory disease.¹ Radiation sensitizers including irinotecan and vorinostat may augment response to ¹³¹I-MIBG and are being evaluated in a randomized trial (NCT02035137), and ¹³¹I-MIBG is being evaluated in a frontline COG pilot trial (NCT01175356). ¹³¹I-MIBG will be studied in Europe in combination with Busulfan/Melphalan ASCR in patients with suboptimal responses to initial therapy, and will be evaluated in COG as part of induction. While tumor norepinephrine transporter (NET) expression appears to correlate with MIBG avidity,⁴⁰ the relationship between NET expression and response to therapy is unknown. Analysis of NET expression and other potential markers of sensitivity to ¹³¹I-MIBG will be important as trials proceed.

Pathway Targeted Therapy

Relatively few somatic mutations have been detected in primary neuroblastoma tumors at the time of diagnosis,^{11,12} though the frequency of mutations is higher at relapse.⁴¹ Activating mutations in the Anaplastic Lymphoma Kinase (*ALK*) are observed in 8% of neuroblastomas at diagnosis,¹³ but may be detected more frequently at relapse.⁴² Inhibitors of *ALK* kinase activity have been developed, including the *ALK*/*MET* inhibitor crizotinib. A study of crizotinib in combination with chemotherapy is underway (NCT01606878), as are early phase trials of next-generation *ALK* inhibitors (NCT01742286 and NCT02097810). Other kinase inhibitors are also in development. Detection of an increased number of *RAS*-*MAPK* mutations in tumors at relapse¹⁶ has increased interest in inhibitors of signaling molecules in this pathway. Several *MEK* inhibitors are being evaluated, and a pediatric trial of trametinib (NCT02124772) has recently begun.

Amplification of *MYCN* remains the most common somatic alteration in neuroblastoma tumors, and multiple approaches to alteration of *MYCN* expression have been explored. Aurora A kinase modulates stability of the *MYCN* protein and activity of the Aurora A kinase inhibitor alisertib (MLN8237) was seen in preclinical models.^{43,44} This agent is being evaluated in combination with chemotherapy in an ongoing trial (NCT01601535). Components of the *PI3K*/*AKT* pathway have also been shown to modulate the stability of the *MYCN* protein and are candidates for further study (reviewed in⁴⁵). In addition, downstream targets of *MYCN* are being evaluated. Ornithine decarboxylase 1 (*ODC1*) is a *MYC* target gene, and *ODC1* is a key regulator of polyamine synthesis within cells. An inhibitor of *ODC1*, α -difluoromethylornithine (DFMO), increases tumor-free survival in preclinical neuroblastoma models.⁴⁶ This agent is being evaluated in combination with chemotherapy in an early phase trial (NCT02030964).

Cell Surface Molecules as Targets for Therapy

The most successful strategy for targeting of cell surface molecules has been ganglioside-directed therapy. In addition to the chimeric antibody dinutuximab, other anti-GD2 antibodies have been developed, including murine and humanized antibodies.(reviewed in⁴⁷) GD2-directed antibodies are being studied with cytokines, cellular immune effectors, other immune modulators, and chemotherapeutics (NCT01757626, NCT00877110, NCT00911560, NCT01711554, NCT01767194). An alternative approach to GD2-directed neuroblastoma therapy makes use of effector lymphocytes modified to express chimeric antigen receptors (CARs). The presence of a GD2-specific extracellular single-chain variable fragment allows for major histocompatibility complex-independent tumor antigen recognition and T cell stimulation. Activity of GD2 CARs has been reported in preclinical models, and initial clinical studies have demonstrated the safety of this approach (reviewed in⁴⁸). Additional GD2 CAR studies are underway (NCT01822652, NCT02107963) or in development. Other cell surface antigens provide alternative targets for CAR T cell therapy for neuroblastoma, including the adhesion molecule CD171 (L1-CAM).⁴⁹ Evaluation of additional cell surface targets is an active area of research.(reviewed in⁵⁰)

Conclusion

Correlative science has been an integral part of neuroblastoma trials for decades, and significant progress in our understanding of the epidemiology and pathobiology of this disease has been gained through the use of well-annotated biobank samples. However, a substantial gap exists between the discovery of molecular drivers of malignant behavior and our ability to translate this knowledge into less toxic, more effective therapy. As we look to the future, it is clear that alterations in DNA sequence in tumor cells alone will not provide all of the information we need to improve outcomes. Instead, we must also take into account data generated through expression profiling, epigenetic analyses, proteomic studies, and host germline alterations to understand and predict the behavior of this malignancy in a comprehensive way. Furthermore, we must develop robust predictive biomarkers to identify patients who will benefit most from components of modern neuroblastoma therapy. Use of a larger number of prognostic factors and biomarkers will provide rationale for therapy tailored to ever-smaller subsets of patients. However, experience suggests that rational therapies cannot be assumed to be effective without being tested in prospectively studies. Design of new-era clinical trials for very small groups of children with neuroblastoma represents the next frontier in investigation.

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Key Points

- Risk-based treatment has led to improved survival for patients with neuroblastoma, but survival for high-risk patients remains poor.
- The prognostic criteria integrated into current risk classification systems have significant limitations, and more robust predictive biomarkers are needed to identify patients who will benefit most from components of modern neuroblastoma therapy.
- Significant progress in our understanding of the molecular underpinnings of the etiology and pathobiology of neuroblastoma has been gained through the use of well-annotated biobank samples.
- A substantial gap remains between the discovery of molecular drivers of malignant behavior and our ability to translate this knowledge into less toxic, more effective therapy.
- Integration of refined treatment stratification based on whole-genome profiles with therapeutics that target the molecular drivers of malignant behavior in neuroblastoma has the potential to dramatically improve the survival of children with high-risk neuroblastoma.

Figure 1.

Probability of event-free survival (EFS) (**A**) and overall survival (OS) (**B**) among 3,352 Children's Oncology Group (COG) patients with high-risk neuroblastoma diagnosed between 1990 and 2010 according to era. 356 patients were diagnosed between 1990 and 1994; 497 were diagnosed from 1995 to 1999; 1,105 were diagnosed from 2000 to 2004, and 1,484 were diagnosed from 2005 to 2010. (Data provided by the COG Statistics and Data Center.)

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Table 1

Current and Future INRG Classification System Variables

INRG Classification System	INRG Classification System II	
INRG Stage	Tumor Genomics	DNA copy number
Age		mRNA expression
Histological category		miRNA expression
Grade of tumour differentiation	Prognostic molecular biomarkers	
MYCN	Therapy-specific predictive biomarkers	
11q aberrations	Germline genotype	
DNA Ploidy	Early response to therapy	