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Recent biologic and genetic advances in neuroblastoma: Implications for diagnostic, risk stratification, and treatment strategies

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ABSTRACT

Neuroblastoma is an embryonic cancer of neural crest cell lineage, accounting for up to 10% of all pediatric cancer. The clinical course is heterogeneous ranging from spontaneous regression in neonates to life-threatening metastatic disease in older children. Much of this clinical variance is thought to result from distinct pathologic characteristics that predict patient outcomes. Consequently, many research efforts have been focused on identifying the underlying biologic and genetic features of neuroblastoma tumors in order to more clearly define prognostic subgroups for treatment stratification. Recent technological advances have placed emphasis on the integration of genetic alterations and predictive biologic variables into targeted treatment approaches to improve patient survival outcomes. This review will focus on these recent advances and the implications they have on the diagnostic, staging, and treatment approaches in modern neuroblastoma clinical management.

Epidemiology and incidence

Neuroblastoma is a developmental cancer of the sympathetic nervous system that is thought to arise during neural crest cell differentiation.[1] The overall incidence is 1 case per 100,000 children in the United States, affecting approximately 700 children per year. Neuroblastoma is the most common cancer in infants and the most common extracranial tumor in all children. Although this cancer represents 8% of all pediatric cancer, it is responsible for 15% of pediatric cancer deaths.[2] The overall survival is 65%, although the majority of patients present with metastatic high-risk disease where survival rates are below 50% despite aggressive surgery and dose-intensified chemotherapy.[3–5] The incidence of neuroblastoma is slightly higher among males than females, and it occurs more frequently in Caucasians from North America, Europe, Australia, and Israel.[6] African–American and Native American children have a higher prevalence of high-risk neuroblastoma and worse event-free survival than European–Americans.[7] Causal factors have not been identified and studies that suggest an association between premature delivery (< 33 weeks), very low birth weight (< 1500 g), or parental occupational exposures remain inconclusive.[8,9] The clinical course is complex, ranging from spontaneous regression in the perinatal period to refractory metastatic disease in older children. This heterogeneity is characterized by many factors such as age at diagnosis, stage of disease, and distinct biologic features of the tumor that predict survival outcomes. There has been significant progress in disease-free and overall survival in neuroblastoma, but treatment challenges remain for patients with refractory and relapsing disease.[10] Effective curative options remain elusive for such patients and they typically endure treatment-related adverse events including subsequent malignancies.

Genetic risk

Although most cases of neuroblastoma are sporadic, rare familial cases provide insight into the genetic etiology. Approximately 1–2% of neuroblastoma cases are familial in an autosomal dominant manner.[11,12] *PHOX2B* and anaplastic lymphoma kinase (*ALK*) genes have been identified as predisposition genes in hereditary cases despite incomplete penetrance.[13] Studies have linked neuroblastoma occurrence as a component of the neurocristopathies that include Hirschsprung's disease, neurofibromatosis, and central hypoventilation syndrome with inherited loss of *PHOX2B*, a regulator of neural crest development.[14] Genome-wide linkage analysis and sequencing of candidate genes identified *ALK* mutations in more than 50% of familial cases at the 2p23–p24 locus, although only about 50% of heterozygous carriers developed neuroblastoma.[12,13] This is likely due to variants in the *ALK* gene or

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linked genes on chromosome 2p23–p24 that may influence disease penetrance. Genetic testing for *ALK* and *PHOX2B* is therefore considered for children with a positive family history of neuroblastoma. Surveillance with screening ultrasound and urinary catecholamine metabolites is recommended for children with these heritable mutations.

In sporadic tumors, *ALK* mutations have been found in somatically acquired neuroblastoma and are associated with poor clinical outcomes in 5–15% of cases.[15,16] *ALK* is an orphan receptor tyrosine kinase in which pathogenic activating mutations have also been found in non-small-cell lung cancer and in anaplastic large-cell lymphoma.[17] *ALK* is now considered an oncogenic driver of neuroblastoma and is a promising novel therapeutic target. Preclinical drug studies of small molecule ALK inhibitors have shown therapeutic potential in neuroblastoma cell lines and in xenografts expressing mutated *ALK*.[18] Early-phase clinical trials examining the antitumor activity, safety, and dosing of targeted inhibition of ALK have had generally positive results and warrant further investigation in neuroblastoma tumors with activating *ALK* mutations.[19]

Genome-wide association studies have also discovered other common predisposing genetic variants that are associated with tumor phenotype and neuroblastoma susceptibility.[20] These findings are important because they suggest that genomic variation may underlie events that initiate tumorigenesis. Susceptibility to low-risk neuroblastoma is associated with single nucleotide polymorphisms (SNPs) within *DUSP12* (at 1q23), *HSD17B12* (at 11p11.2), *DDX4*, and *IL31RA* (both at 5q11.2) and correlate with less aggressive disease.[21] SNPs at *FLJ22536*, *FLJ44180* (6p22), *CASC15*, *CASC14*, *BARD1*, *LM01, HACE1*, and *LIN28B* have been found in tumors of patients with high-risk disease and indicate susceptibility to aggressive neuroblastoma.[22–26] DNA copy number variants also represent a source of genetic diversity. Inherited copy number variation at 1q21.1 is associated with neuroblastoma tumors and the *NBPF23* gene has been implicated in tumorigenesis.[23] Common variant polymorphisms may work additively to activate neuroblastoma tumor initiation. Several of these DNA variations have been found to influence gene and protein expression to promote tumorigenesis and tumor progression. This is exemplified by compelling evidence that *BARD1* is the most significant genetic contributor to neuroblastoma risk and may promote tumor growth and progression.[25,26] Additionally, because *BARD1* has been found to interact with and stabilize the Aurora kinases, a potential therapeutic target, further studies are focusing on the translational potential of cancer susceptibility genes.[27] Elucidation of the underlying pathogenic nature of these and other genomic abnormalities will identify biologic pathways that control tumor initiation and progression, and can be targeted in patients with refractory disease.[28]

Genetic variation and disparities in survival have been observed in African–American children with high-risk neuroblastoma.[29] African–American ancestry is associated with high-risk disease and lower event-free survival compared to Caucasian children.[7] This has been linked to genetic variation at known high-risk neuroblastoma susceptibility loci identified by genome-wide association studies.[20] Several known SNPs found in high-risk neuroblastoma (*LMO1, LINCOO340*) were found to have higher risk allele frequencies in African–American children.[29] Such findings highlight the key role that genomic variations may have in predicting risk group and clinical outcomes in children with high-risk disease.

Amplification of the *MYCN* oncogene ($\geq$ 10 copies) on chromosome 2p24 is the most consistent genetic alteration in neuroblastoma and is a reliable indicator of clinically aggressive disease.[30] Approximately 22% of tumors have *MYCN* amplification and of these, 90% are associated with poor clinical outcomes. *MYCN* transcription signals regulate proliferation and differentiation of the developing nervous system and neuroblastoma tumors.[31] *MYCN* is a major oncogenic driver of neuroblastoma and although no clinical trials have directly targeted the oncogene, inhibition of signaling pathways that deregulate *MYCN*, such as aurora kinase A and BET bromodomain signaling, has shown antitumor activity in preclinical studies.[1,32]

α-Thalassemia/mental retardation syndrome X-linked (*ATRX*) mutations are also among the most common genetic lesions in neuroblastoma.[33] *ATRX* functions in neural development and differentiation, though the precise mechanistic role is unknown. *ATRX* mutations are found in approximately 17% of children with neuroblastoma between 18 months and 12 years, and in 44% of patients older than 12 years with Stage 4 disease.[33] Conversely, *ATRX* mutations are not found in infants and younger children. This suggests that analysis of the *ATRX* gene may provide age-group-specific insight into the propensity of older children to present with more aggressive tumors. Further studies are needed to determine how genetic analysis of *ATRX* status could potentially identify patients likely to develop neuroblastoma.

Several recurrent segmental chromosomal alterations have been identified in neuroblastoma tumors and are the strongest predictors of neuroblastoma relapse.[34] Loss of heterozygosity (LOH) of chromosome 1p predicts poor survival outcomes despite other favorable biologic predictors.[35] LOH at 1p reliably predicts patients at high-risk of death in the subgroup without *MYCN* amplification or in low-stage disease.[35] Gain at chromosome 17q occurs commonly with 1p loss and is also associated with poor outcomes.[36] Importantly, 17q gain is the most frequent segmental chromosomal abnormality in neuroblastoma tumors and is associated with poor outcomes, older age, *MYCN* amplification, and diploidy.[36,37] Loss of chromosome 11q is also a recurrent segmental chromosomal alteration, present in up to 40% of neuroblastoma tumors.[38] 11q LOH is an important indicator of risk because it is associated with poor outcomes in the subset of patients with low-stage disease without *MYCN* amplification (Table 1). Comprehensive segmental genomic analyses have shown that in patients without *MYCN* amplification, a segmental chromosomal alteration is associated with a high rate of relapse and worse outcomes compared to patients without segmental anomalies.[39,40] Given this, a segmental genotype may provide valuable prognostic information, particularly in patients without *MYCN* amplification, and may reflect an underlying oncogenic advantage.[41,42]

Pathology and biologic features

Neuroblastoma tumors arise from primitive neuroectodermal cells of the developing sympathoadrenal nervous system.[43] The hallmark of neuroblastoma tumors is clinical heterogeneity ranging from spontaneous regression in perinatal cases and refractory metastatic disease in older children. The precise molecular event

Table 1
Common prognostic segmental chromosomal alterations in neuroblastoma tumors without MYCN amplification.

Chromosome	Segmental alteration	4-Year EFS (%)	4-Year OS (%)
1p	Deletion	55 ± 3.7	72 ± 3.3
	None	63 ± 2.9 ($P = 0.06$)	79 ± 2.6 ($P = 0.11$)
11q	Deletion	42 ± 3.8	65 ± 3.9
	None	75 ± 3.0 ($p < 0.0001$)	88 ± 2.4 ($P < 0.0001$)
17q	Gain	49 ± 4.7	72 ± 4.3
	None	75 ± 3.6 ($P = 0.0002$)	86 ± 2.9 ($P = 0.0001$)

Abbreviations: EFS = event-free survival; OS = overall survival.
Adapted with permission from Schleiermacher et al.[34]