Clinical Perspective

Genetic Prognostic Markers in Neuroblastoma

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Abstract

Neuroblastoma is the most frequent pediatric extracranial tumor. It arises from embryonic neural crest cells and exhibits significant heterogeneity of clinical presentation and biologic behavior. Despite the application of aggressive treatments and multiple risk stratifications, prognosis of advanced stage of neuroblastoma still remains poor. So it is still a challenge in its prognostic evaluation to both clinicians and basic scientists and it is necessary to explore more molecular factors that correlate with outcome of this disease and develop a statistical predictor of survival for neuroblastoma patients. In this review, we summarize recent findings of chromosome alteration, gene expression, microRNA profiles and gene methylation status in neuroblastoma as well as their implications for predictors of neuroblastoma prognosis.

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Introduction

Neuroblastoma is a malignant tumor of peripheral sympathetic nervous system.[1] It derives from neural crest cells whose differentiation was terminated during embryo development. It is most commonly found in the adrenal medulla or along the sympathetic chain.[2] Neuroblastoma is one of the most common solid malignant tumors in children and the most frequently diagnosed neoplasm during infancy.[3] It affects about 10.5/10 million children younger than 15 years of age per year.[1] Recently one epidemiology study showed the incidence rate for neuroblastoma was about 1.6 per 10,000 European children younger than 15 years of age per year.[4] It accounts for 7-10% of all malignancies of childhood and about 15% of all pediatric cancer deaths.[1-2,5-6]

Neuroblastoma is extremely heterogeneous and its clinical presentations and behavior range from spontaneous regression to extensive hematogenous metastases.[7] Neuroblastoma in fetus and infancy are capable of spontaneous regression and differentiation into benign tumors without needs of any clinical therapies.[3,7] However, there remains a large number of neuroblastoma ($\geq$ 50%) presenting a rapid and progressive disease.[8] Furthermore, metastasis frequently occurs in near 70% of neuroblastoma patients at the time of primary diagnosis.[9] Prognosis of the advanced stage of disease still remains poor despite aggressive treatment regimens. In the past few years, the outcome of patients with a high-risk clinical phenotype has improved only modestly, with long-term survival still less than 40%.[10,11] Both genetic and environmental factors may contribute to neuroblastoma oncogenesis and its clinical feature[12-16]. However, little is known about its underlying mechanisms of carcinogenesis and it continues to be a great challenge to physicians as well as basic scientists. Thus, it is necessary to understand more biologic features of neuroblastoma and to develop novel and more effective therapies to improve the outcome for neuroblastoma patients.

Risk Stratification in Neuroblastoma

Current therapy protocols for neuroblastoma are based on its risk stratification including clinical and biologic features.[1-2,17] Previously, various approaches to risk classification and treatment stratification of neuroblastoma were applied to clinical diagnosis and treatment throughout the world.[18] To date, the International Neuroblastoma Risk Grouping Staging System (INRG) has been used to classify neuroblastoma from patients in a uniform manner all over the world (**Table 1**).[18] The system includes the criteria INRG stage, age, histological category, grade of tumor differentiation, the

status of the *N-Myc* oncogene, Chromosome 11q status and DNA ploidy. According to this stratification, there are low, intermediate, and high risk subgroups in neuroblastoma.[18] Current risk-based therapy is effect in patients with low or intermediate risk disease but far from satisfactory in the advanced stage of disease.[17,19] Therefore, more accurate predictors of prognosis for neuroblastoma patients should be involved in risk stratification and improve intensive therapies for neuroblastoma patients in the high-risk subgroup.

Table 1. International Neuroblastoma Risk group (INRG) consensus pretreatment classification schema.

INRG stage	Age (Months)	Histological category	Grade of tumor differentiation	N-myc	11q aberration	Ploidy	Pretreatment risk group
L1/L2		GN maturing; GNB intermixed					A Very low
L1		Any except GN maturing or GNB intermixed		NA			B Very low
				Amp			K High
L2	< 18	Any except GN maturing or GNB intermixed		NA	No		D Low
					Yes		G Intermediate
	≥ 18	GNB nodular; neuroblastoma	Differentiating	NA	No		E low
					Yes		
			Poorly differentiated or undifferentiated	NA			H Intermediate
				Amp			N High
M	< 18			NA		Hyperdiploid	F Low
	< 12			NA		Diploid	I Intermediate
	12 to < 18			NA		Diploid	J Intermediate
	< 18			Amp			O High
	≥ 18						P High
MS	< 18			NA	No		C Very Low
					Yes		Q High
				Amp			R High

Amp, amplified; NA, not amplified; EFS, event free survival; GN, ganglioneuroma; GNB, ganglioneuroblastoma.
L1, localized tumor confined to one body compartment and with the absence of image-defined risk factors; L2, locoregional tumor with presence of one or more image-defined risk factors; M, distant metastatic disease (except stage MS); MS, metastatic disease confined to skin, liver or bone marrow in children < 18 months of age.
Very low risk= 5-year EFS>85%; Low risk=5-year EFS 75-85%; high risk= 5-year EF < 50%.

Genetic Prognostic Markers

Neuroblastoma is characterized by high genetic heterogeneity.[2] Accumulating evidence suggested that several genetic variations such as *N-Myc* amplification, 1p and 11q deletion are associated with neuroblastoma initiation, progression including multidrug resistance, invasion and metastasis as well as prognosis.[2] With the application of high-resolution whole-genome methods including microarray-based technologies, novel genetic variations are identified and implicated in neuroblastoma prognosis.[20-21]

Chromosomal alterations

Genomic imbalance in neuroblastoma

Genomic imbalance, ranging from imbalance of entire chromosomes to submicroscopic rearrangements, has been found in several pediatric diseases including neuroblastoma.[22-24] So identification and characterization of genomic imbalance will be effective to explore the etiology and prognosis of these diseases. *N-Myc* was the first identified oncogene associated with neuroblastoma.[25] Amplification of *N-Myc* contribute to multiple aggressive phenotypes including rapid progression and poor outcome.[20,26-27] To data, several genomic variations have

been implicated as novel prognostic markers for neuroblastoma patients. A couple of experiments suggested that chromosome 1q gain is associated with poor outcome in neuroblastoma patients. 1q gain was implicated as an independent predictor of decreased overall survival based on a whole-genome DNA copy number analysis in 493 neuroblastoma patients.[28] Pezzolo et al. found that 1q22qter gain was exclusively detected in stroma-poor localized resectable neuroblastoma patients who underwent relapse or progression.[29] On the contrary, 7p gain was a common chromosome variation in more than 30% of patients with complete remission, which predicts a better event free survival.[29] Gain of chromosome 17q is considered to be another prognostic marker in neuroblastoma patients without *N-Myc* amplification or 11q deletion.[30-32] Patients with 17q gain will have a high risk of relapse and poor outcome.[30-32]

Single nucleotide polymorphism (SNP)

SNP is a single nucleotide variation in a DNA sequence between different members of one species.[33] Specific SNPs are associated with various human diseases including neuroblastoma.[34-35] One genome-wide association study was performed in 1,032 neuroblastoma patients and 2,043 controls, which revealed that neuroblastoma patients with homozygous for three risk alleles at chromosome 6p22 were more likely to develop metastatic diseases.[36] Interleukin-6 SNP genotype, rs1800795 SNP [-174 IL-6 (G > C)] represents a novel and independent prognostic marker for both event free survival and overall survival in high-risk neuroblastoma.[37] c.1810C>T polymorphism of NTRK1 gene that encodes tyrosine kinase receptor for neurotrophins is also associated with poor outcome in neuroblastoma patients.[38] Five year overall survival was 77.6% in patients with c.1810CC while 26.3% in those with c.1810CT and c.1810TT.[38] Furthermore, 5-year event free survival for c.1810CC (73.8%) was also higher than that for c.1810CT and c.1810TT (26.3%).[38]

Gene expression profiling of neuroblastoma

Different outcome in patients with neuroblastoma may reflect various genetic and biological characteristics which are caused by abnormal gene expression profiles.[20-21] Therefore, detection of gene expression will explore several gene-expression-based classifiers and improve prognosis prediction in neuroblastoma patients. Several whole genome gene expression profiles were performed to explore some genes related to prognostic prediction. Wei et al. identified 19 genes which were implicated in accurate prediction of prognosis in neuroblastoma patients by using gene expression profiling and artificial neural networks.[39] A tumor-specific cDNA microarray comprising 5340 genes revealed a prognostic gene expression signature for intermediate risk neuroblastomas.[40] Schramm et al analyzed the expression data of 68 neuroblastomas with different risks by support vector machines (SVM-rbf), prediction analysis of microarrays (PAM), k-nearest neighbors (k-NN) algorithms or multiple decision trees. A classifier of 39 genes was identified and gene expression data had a higher accuracy of neuroblastoma prognosis compared with conventional risk stratification described above.[41] Oberthuer et al. also confirmed that integration of gene expression-based classifiers might improve prognosis in neuroblastoma.[42]

MicroRNA in neuroblastoma

MicroRNAs are a large number of small non-coding RNAs that bind to complementary sequences in the 3'UTRs of target mRNA and block the translation or degrade the respective mRNA.[43] As post-transcriptional regulators, MicroRNAs are involved in multiple biological processes including cell metabolism, cell cycle and survival as well as cell apoptosis. A down-regulation of microRNAs may contribute to multiple human diseases such as schizophrenia, heart diseases and malignant tumors.[43-45] Several microRNAs have been found to have links with the outcome of neuroblastoma patients.[43] Chen et al. identified several microRNAs differentially expressed in different prognostic subtypes of neuroblastoma.[46] Usually, most of them have down-regulation induced by *N-Myc* amplification in neuroblastoma high-risk subgroup.[46] Combined with *N-Myc* amplification, microRNAs down-regulation predicts a poor prognosis in neuroblastoma patients.[46] Welch et al. found miRNA-34a that targets E2F3 mRNA directly and blocks its protein expression had a low level expression in unfavorable primary neuroblastoma compared to normal adrenal tissue.[47] Another study showed high expression of MRHG1 was significantly associated with high-risk groups of neuroblastoma, which was caused by dysregulation of OncomiR-1 including miR-17, miR-18a, miR-19a, miR-20a and miR-92a.[48] In another study, miR-18a and miR-19a have been found to target and repress the expression of estrogen receptor-alpha (ESR1), and high ESR1 expression correlates with increased event-free survival in NB patients and favourable disease outcome.[49] Recently, other 13 microRNAs were indentified as outcome predictors since their expressions were significantly different between long and short surviving patients with neuroblastoma, and the microRNA/T-UCR network may contribute to the pathogenesis and prognosis of neuroblastoma.[50]

DNA methylation profiling of genes in neuroblastoma

DNA methylation is a process of the conversion of the cytosine to 5-methylcytosine, which is performed by DNA methyltransferases at CpG sites in the DNA sequence.[51] It is mainly involved in embryonic development and gene expression in postnatal development.[51] Several abnormal DNA methylations have been also associated with neuroblastoma[52-55] Hypermethylation of the cyclin-dependent kinase inhibitor CDKN2A (p16) led to loss of p16 expression, which was correlated with short survival of neuroblastoma patients.[52] Patients with serum DCR2 methylation showed significantly poor 5-year event free survival (43% vs. 84% in patients without DCR2 methylation).[53] High-methylated CpG in the caspase-8 gene (CASP8) was correlated with *N-Myc* amplification and resulted in progressive behavior in neuroblastoma patients in high-risk subtype.[54] RASSF1A, another tumor inhibition gene, is significantly hypermethylated in 94% of neuroblastoma patients with poor event free survival.[55]

Conclusions

There exist varieties of genetic/genomic alterations including chromosome variation, gene expression alteration, and microRNA deregulation as well as demethylation of genes in neuroblastoma. Complex networks and its mechanisms among these abnormalities may contribute to neuroblastoma oncogenesis and determine its clinical behavior. Therefore, with more identification of more of these genetic markers related to prognosis of neuroblastoma, more accurate risk stratification will be developed. The survival will be improved and risk-based therapies are expected to be effect in neuroblastoma patients particularly those with advanced stage disease in future clinical trials.

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