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Overview

Loss of Heterozygosity in Neuroblastomas—an Overview

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Although previous studies have demonstrated a relatively high incidence of loss of heterozygosity (LOH) on chromosomes 1p, 11q and 14q in neuroblastoma, it is unclear whether LOH occurs specifically on these chromosomes or not. It might be due to the lack of allelotyping of neuroblastoma. When we assessed all 22 autosomes and chromosome X for LOH in 81 cases of neuroblastoma using 43 polymorphic DNA markers, a high incidence of LOH (> 30%) was observed on three chromosomal arms, 2q (30%), 9p (36%) and 18q (31%). Moreover, 9p LOH in the tumours showed statistically significant association with advanced stage of the disease and poor prognosis. Therefore, tumour suppressor genes on chromosomes 2q, 9p and 18q could be involved in the genesis and/or progression of neuroblastoma. Particularly, the gene on chromosome 9p may be associated with progression of neuroblastoma. © **1997 Elsevier Science Ltd.**

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NEUROBLASTOMA IS a common childhood malignancy of the sympathetic nervous system showing considerable variability in clinical evolution. It is usually classified by the stage of disease (I + II + IVS, III and IV) and the age of patients at diagnosis (more or less than one year old), since these factors are known to be closely associated with the prognosis of patients. In Japan, a mass screening programme for infants at the age of 6 months has been performed since 1973 and the patients found by this screening have been mostly classified to the early stage of the disease [1, 2].

Although various studies have been done to elucidate the molecular mechanisms for the genesis and progression of neuroblastoma, molecular genetic events involved in the genesis and/or progression of neuroblastoma are not fully understood. *MYCN* oncogene amplification has been known as the most significant prognostic factor of neuroblastoma [3, 4]. However, even in stage IV patients, occasional cases of advanced disease without *MYCN* amplification are noted, suggesting the presence of other genetic events involved in the progression of neuroblastoma [5]. Alterations in several tumour suppressor genes, such as *p53*, *RB*, *NF1* and *p16*, are known to be rare in neuroblastoma (Table 1) [6–12]. Although a relatively high incidence of loss of heterozygosity (LOH) on at least three chromosome arms, 1p, 11q and 14q, has been reported in neuroblastoma (Table 1) [13–20], it is still unknown whether LOH occurs specifically on these chromosomes or not. This might be due to the lack of allelotyping of neuroblastoma. Therefore, we examined all 22 autosomes and chromosome X for LOH in 81 cases of neuroblastoma using 43 polymorphic DNA markers. A high incidence of LOH (> 30%) was observed on three chromosome arms, 2q (30%), 9p (36%) and 18q (31%). Chromosomes 1p, 11q and 14q were lost in 26%, 24% and 22%, respectively [21]. Frequencies of LOH on other chromosome arms were less than 13%. Therefore, besides LOH on chromosomes 1p, 11q and 14q, LOH on chromosomes 2q, 9p and 18q also occurs frequently in neuroblastoma.

Several studies have shown the association of chromosomal deletions with biological aspects. Chromosome 1p deletions in neuroblastoma are significantly correlated with the progression of neuroblastoma. Furthermore, recently, Takeda and associates [20] and Schleiermacher and associates [19] proposed that chromosome 1p could contain two tumour suppressor genes closely associated with biologically distinct subtypes of neuroblastoma. The patients with interstitial deletions which encompassed a small region in 1p36 were mostly less than one year of age, were frequently found by the mass screening programme for infants, had a tumour of non-adrenal origin and were mostly classified as early stage

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Table 1. *Genetic alterations in neuroblastoma*

Genetic alterations	Incidence	Reference
Oncogene abnormalities		
MYCN amplification	67% (stage IV)	1
	48% (stage IV)	15
Tumour suppressor gene abnormalities		
p53 mutation	2%	8
NF1 mutation	two cell lines	9
p16 deletion	0/18	12
Loss of heterozygosity		
Chromosome 1p	28%	13
	22%	14
	32%	15
	28%	17
	37%	18
	20%	20
Chromosome 2q	30%	21
Chromosome 9p	36%	21
Chromosome 11q	5%	15
	32%	18
Chromosome 14q	50%	14
	20%	15
	22%	18
	40%	16
	24%	21
Chromosome 18q	31%	21

of the disease. In contrast, patients with terminal deletion which encompassed the region from chromosome 1p32 were mostly over 1 year of age, were frequently accompanied by *MYCN* amplification and were rarely classified as early stage. However, occasional cases with 1p32-qter LOH in early stage were also noted. Takayama and associates [16] and Srivatsan and associates [18] indicated that deletion of chromosome 14q may not correlate with the aggressiveness of neuroblastoma. However, it has also been reported that LOH for chromosomes 1p and 14q occurred in patients with advanced stage of the disease [15]. In addition, Srivatsan and associates [16] also suggested that LOH on chromosome 11q may play an important role in metastasis of neuroblastoma. To date, it cannot be concluded which chromosomal deletions are most important for the genesis and progression of neuroblastoma. We have further examined the association between clinicopathological findings and the allelotype of neuroblastoma and found that 9p LOH are associated with advanced stage of the disease and poor prognosis, whereas LOH on other chromosomes do not show association with any of these parameters [8]. We did not find evidence for correlation between 1p, 2q, 11q, 14q and 18q LOH and progression of neuroblastoma in our studies [21].

Recently, we found a homozygous deletion on chromosome 2q33 in a human small cell lung carcinoma cell line by arbitrarily primed PCR genomic fingerprinting and detected a high incidence of LOH on chromosome 2q in several human cancers [22]. These results indicate that a tumour suppressor gene(s) on chromosome 2q is involved in the genesis and/or progression of several human cancers. Two candidate tumour suppressor genes, the *p15* and *p16* genes, have been identified and mapped closely to the *IFNB1* locus on chromosome 9p21 [23–26]. Therefore, these genes might be target tumour suppressor genes associated with the progression of neuroblastoma. However, using Southern blot analysis, we detected no homozygous deletions in these tumours. Further analysis should provide information on whether the *p15* and *p16* genes are involved the aggressiveness of neuroblastoma. The *DCC* gene on chromosome 18q is one of several genes found to be altered during colorectal tumorgenesis [27]. Recent studies indicate that *DCC* plays a role in both epithelial and neural cell differentiation [28]. Thus, it is possible that the *DCC* gene is involved in the genesis and/or progression of neuroblastoma.

In conclusion, besides LOH on chromosomes 1p, 11q and 14q, LOH also occurs relatively frequently on chromosomes 2q, 9p and 18q in neuroblastoma, indicating the involvement of multiple tumour suppressor genes in the development of neuroblastoma. In particular, one tumour suppressor loci located on chromosome 9p could be involved in the progression of neuroblastoma. Therefore, further studies are now in progress in our laboratory to identify target tumour suppressor gene(s) on chromosome 9 in neuroblastoma.

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