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Original Paper

Prognostic Relevance of Genetic Alterations in the p32 Region of Chromosome 1 in Neuroblastoma

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Thirty-six neuroblastomas were analysed for chromosome 1p alterations and their prognostic relevance. In 72% (26/36) of the patients, 1p alterations were identified in the tumours using 24 polymorphic loci ranging 1p22–1p36.3. LOH was identified in 25 children, and in 10 additional allelic imbalance was identified. In 1 child allelic imbalance was the sole alteration. Imbalance was termed as gain in intensity of one allele with or without reduction of the second allele (< 50%). The imbalance was identified in adjacent regions to the LOH. Two distinct regions of LOH were identified: 1p36.1–p36.3 and 1p31–p32. The common imbalance regions overlapped the common LOH regions. The children with LOH and imbalance had improved survival (100%) compared to the children with LOH only (26%) after 48 months of follow-up. The imbalance had an advantageous effect that is reflected by the improved outcome in children with other unfavourable clinical features. © **1997 Published by Elsevier Science Ltd.**

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INTRODUCTION

NEUROBLASTOMA IS a heterogenous tumour, resulting in diverse clinical behaviour and outcome [1]. The most frequent genetic alteration observed in neuroblastoma is deletion of the short arm of chromosome 1 [2, 3]. Cytogenetic and molecular studies analysing chromosome 1p have identified common regions for the deletions. They have also revealed that the genetic alterations are not exclusively simple 1p deletions resulting in loss of heterozygosity (LOH). Replacement of 1p by material from 17q has been shown to be a frequent mechanism for LOH [4, 5]. A reciprocal translocation (1;15)(p36.2;q24) may have caused duplication of the 1p material [6]. The most common deleted region has been defined to the chromosomal bands 1p36.2–p36.3 [7–9]. However, recent studies have shown that there are at least two more different deleted chromosomal regions in patients with neuroblastoma [10, 11].

The issue of prognostic relevance of 1p LOH has been discussed in all LOH analyses. There are some reports which claim that there is no prognostic relevance of 1p LOH [12, 13]. In others, an association between 1p LOH and decrease in survival has been demonstrated [9, 14]. Recently, Caron and associates [15], using multivariate analysis, have shown that 1p LOH is a strong prognostic factor, independent of age and stage. These discrepancies can be explained by the difference in the location of the polymorphic markers used. We studied 36 neuroblastomas for LOH of 1p markers distributed at 1p22–1p36.3.

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MATERIALS AND METHODS

Tumour and constitutional samples were obtained from 36 neuroblastoma patients treated at the Pediatric Hematology Oncology, Schneider Children's Medical Center of Israel. 30 patients were classified according to Evan's staging [16] as III–IV and 6 as stages I–II. Twenty-eight were over and 8 under 1 year of age. High molecular weight DNA was extracted from matched tumour and constitutional samples using the salting out method [17].

A panel of 24 polymorphic markers along the short arm of chromosome 1 (1p22–1p36.3) were used for LOH analysis. The polymorphic markers consisted of: 2 minisatellites (*D1S76, D1S80*), 15 microsatellites (*D1S228, D1S199,*

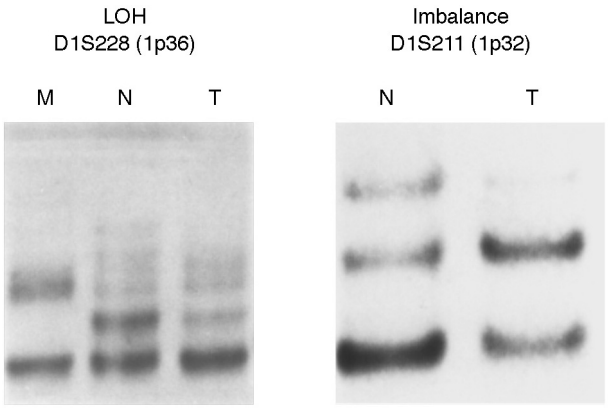

Figure 1. LOH and imbalance identified in a neuroblastoma patient. LOH of the upper allele identified with *D1S228* and imbalance of the upper allele identified with *D1S211* in different regions of chromosome 1 in the same neuroblastoma patient. M, mother; N, normal tissue; T, tumour.

D1S247, D1S513, D1S201, D1S255, D1S193, D1S211, D1S427, D1S200, D1S220, D1S209, D1S198, D1S216, D1S207) and 7 RFLP/VNTR probes (*D1Z2, PND, D1S7, ALPL, D1S57, L-MYC, D1S13*).

Evaluation of intensity was performed using densitometric analysis with Quantity One® software (PDI). LOH was defined as a reduction of > 50% of the intensity; imbalance was defined by a gain of intensity of > 60% of one allele with or without a reduction of intensity of < 50%.

RESULTS

1p alterations

1p alterations were identified in 72% of the children (26/36). Two different types of alterations were identified: LOH and imbalance (Figure 1). Fifteen children had LOH only, 10 demonstrated allelic imbalance with LOH in adjacent regions, and 1 had only allelic imbalance.

Two distinct LOH regions could be identified: NB-1 was defined to 1p36.1–p36.3 by microsatellites *D1S247–D1S80*. NB-2 was defined to 1p31–p32 by *D1S209–D1S211*.

In the cases with imbalance, two distinct regions could also be identified: IM-1 was defined to 1p34–p36.1 by microsatellites *D1201–D1S199*. IM-2 was defined to 1p32 by microsatellites *D1S427–D1S211*. An overlap exists between the defined chromosomal regions for the LOH and imbalance.

Both the groups with LOH only and LOH with imbalance were subjected to progression-free and survival analyses, estimated by Kaplan–Meier and the Mantel–Cox test. Progression-free survival rates at 48 months were 90% and 26% for the LOH with imbalance and LOH only groups, respectively ($P = 0.004$). Survival rates at 48 months were 100% and 26% for the LOH with imbalance and LOH only groups, respectively ($P = 0.0009$).

DISCUSSION

In concordance with previous studies, we have identified two distinct regions for LOH. These regions are similar to those published by Schleiermacher and associates [10] and Takeda and associates [9]—1p36 and 1p32. In contrast to others, we have identified an additional 1p alteration, allelic imbalance. The imbalance could also be defined in two distinct regions—1p34-36.1 and 1p32. The different regions for LOH and imbalance overlap.

Since almost all the children (10/11) demonstrating allelic imbalance also had LOH in adjacent regions, we assumed that the improved survival of this group compared to the children with LOH only is due to the gain of genetic material identified as allelic imbalance. The imbalance could have been caused by several alternative mechanisms: partial triploidy, duplication and genetic heterogeneity of LOH, all leading to the same molecular manifestation. Even though 7/9 (78%) of the children with imbalance had a triploid DNA content, we identified LOH in all of them, so the imbalance may reflect regions that have not undergone LOH. This reflects a regional gain. Noteworthy is the fact that this regional gain is parallel to the identified distinct regions of deletion in the LOH group of children. Duplication of 1p material has been reported at 1p36.1–p36.2 [6, 9] as a part of the process resulting in a reciprocal translocation. An intact suppressor locus may be included within the duplicated region, and thus have increased transcription and translation. Genetic heterogeneity of LOH may also lead to imbalance if only a subpopulation of the tumour cells have undergone LOH. In this case, the tumour partially retains both copies of a putative tumour suppressor gene, and may thus retain normal activity. The advantageous effect of the imbalance is reflected by the improved outcome even in children with other unfavourable clinical features. In all three suggested mechanisms, a dosage effect exists, probably resulting in favourable prognosis.

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