Loss of Heterozygosity at 19q13.3 Is Associated with Locally Aggressive Neuroblastoma

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ABSTRACT

A genome-wide allelic analysis of neuroblastoma (NB) revealed a previously undescribed increased incidence of loss of heterozygosity (LOH) on chromosome arm 19q13 primarily affecting stages 3 and 4N disease. Further allelic analysis of chromosome 19q13 in a cohort of 116 NB patients using 17 polymorphic microsatellite markers identified the shortest common region of loss between D19S606 and D19S112 at 19q13.3. In some cases, clonal LOH at 19q13 was acquired during the course of disease, and deleted clones remained after cytotoxic therapy. In multivariant analysis, 19q13 LOH was associated with overall survival in local-regional International Neuroblastoma Staging System stages 1, 2, and 3 patients and was specifically present in tumors at the site of recurrence.

INTRODUCTION

NB is a pediatric cancer that arises from precursor cells of the peripheral sympathetic nervous system. It is clinically heterogeneous with at least three well-recognized patterns of disease: (a) infants with widespread disease that can spontaneously regress without medical intervention; (b) systemic disease with widespread metastasis that responds to cytotoxic therapy but frequently becomes resistant to medical treatment with poor outcome, mainly because of distant metastasis; and (c) LR NB characterized by lack of distant metastases. Most patients with LR NB have a good prognosis without cytotoxic therapy; however, some LR tumors will recur locally and often become resistant to cytotoxic therapy. These patients finally succumb to uncontrollable local disease.

We performed a genome-wide allelic analysis to identify clinically useful markers of disease progression for each group of NB. This analysis revealed a previously undescribed region of LOH for NB on chromosome arm 19q. An expanded study of 19q LOH in a large sample of NB cases showed that 19q13 LOH occurred primarily in a defined subgroup of high-risk NB patients with propensity for local recurrence.

MATERIALS AND METHODS

Patient Materials and Clinical Characteristics. One hundred fifty-seven NB tumors from 116 patients managed at Memorial Sloan-Kettering Cancer Center, including 10 patients with INSS (1) stage 4s, 45 patients with LR disease (INSS stages 1, 2, 3), and 61 patients with stage 4 were analyzed. Multiple tumor samples obtained over time or from multiple sites at the same surgical procedure were available from 18 patients. All samples were evaluated histologically, and only specimens with >50% tumor cell content were studied, most with >80% of tumor cells. Standard treatment consisted primarily of surgical resection for LR disease and stage 4s disease (2) and the use of an intensive multimodality regimen for stage 4 (3).

LOH Analysis. Genomic DNA from frozen tumors, bone marrow, and peripheral blood was extracted using standard procedures. Polymorphic microsatellite loci were identified in the Genome Database, and fluorescently labeled primers were obtained from Research Genetics (Birmingham, AL). Map locations were taken from the Lawrence Livermore National Laboratories for chromosome 19 as a primary source. Additional information was taken from NIH Genemap99 for chromosome 19q. Marshfield Clinic genetic map, The University of Southampton genetic maps, and maps published previously from Smith et al. (4). Allelic analysis was performed as described previously (5).

Statistics. The association between event-free survival, defined as relapse, and survival, defined as the time to death or last follow-up and clinicobiological variables, was assessed using the log-rank test (6). Those factors that were potentially predictive of event-free survival and overall survival were entered into a multivariate analysis using the Cox proportional hazards model (7). Survival curves were generated using the method of Kaplan and Meier (8). All statistical calculations were performed using S-Plus 2000 (Mathsoft, Inc., Seattle, WA).

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3 The abbreviations used are: NB, neuroblastoma; LR, local-regional; LOH, loss of heterozygosity; INSS, International Neuroblastoma Staging System; SRO, shortest region of overlap.

4 Internet address: http://greengenes.lhln.gov/genome-bin/.
6 Internet address: http://research.marshfieldclinic.org/genetics/MapMarkers/maps/MapsFrames.html.
7 Internet address: http://cedar.genetics.soton.ac.uk/public_html/index.html.

Allelic Analysis of Chromosome Arm 19q in NB. Allelic analysis of chromosome arm 19q was performed in 157 tumors from 116 patients using eight polymorphic microsatellite markers, D19S927, D19S254, D19S180, D19S907, D19S606, D19S412, D19S582, and D19S75, spanning the entire arm 19q, and D19S247 at 19p13 (Fig. 1). Seventeen patients (10%) had 19q13.3 LOH including 1 of 10 (10%) stage 4s, 8 of 45 (17%) LR tumors (INSS stages 1, 2, 3), and 8 of 61 (13%) stage 4 patients. There was a high frequency of 19q LOH in large bulky tumors with lymph node involvement but without metastasis to bone or bone marrow. For LR tumors, 19q13 LOH was most prevalent in stage 3 patients (8 of 15; 53%; Fig. 2). Among stage 4 patients, 6 had no bone or bone marrow metastasis and were subclassified as stage 4N. Of these 6 stage 4N cases, 4 had 19q13 LOH.

Mapping of Losses at 19q. Further analysis to determine the SRO for LOH at 19q was performed in 60 tumors adding eight microsatellite markers on 19q13 between D19S75 and D19S907 (Fig. 3). These tumors included all stage 3 LR and tumors that presented local recurrences. No additional cases with loss were detected. Although there is some uncertainty as to the relative position of some markers, the highest frequency of loss occurred in the interval from D19S412 to D19S247. A common region of loss could be assigned between D19S606 and D19S112 at 19q13.3, overlapping the narrowest region of loss defined recently in gliomas by Smith et al. (4). A few examples of interstitial retention of heterozygosity were observed and could represent homozygous deletions; however, this requires further study.

Clinical Correlations. During the last 12 years at Memorial Sloan-Kettering Cancer Center, patients with LR NB, whether newly diagnosed or treated previously, were managed conservatively and initially treated with surgery only. The excellent survival obtained in this cohort of patients (2) suggested that LR NB tumors generally had low-risk biology. However, a small subset of this group developed progressive disease requiring cytotoxic therapy. A significant correlation was found between 19q13 LOH and aggressive LR disease. Among the 45 LR NB tumors studied, only seven patients have died of disease. Tumor samples from five of these seven patients (71%) had 19q13 LOH. Multivariate analysis of the prognostic factors (including age, stage, histology, ploidy, MYCN, 1p LOH, 9p LOH, 11q LOH, 14q LOH, and 19q13 LOH) showed that diploidy and 19q13 LOH were the best two independent prognostic variables for overall survival in this cohort (P = 0.000255). There was a relative risk 0.0838 for patients with triploid DNA index for dying of disease (95% confidence interval, 0.013–0.538) and a relative risk 5.5695 for dying of disease with 19q13 LOH (95% confidence interval, 1.15–26.89). Six of the seven cases that relapsed without 19q13 LOH were diploid tumors, and five of the six cases with 19q13 LOH that did not relapse at the primary site were near-triploid. Ploidy and 11q LOH were most significant in predicting progression among LR NB (P = 0.00036). There was a relative risk 0.141 for progression of triploid tumors (95% confidence interval, 0.054–0.368) and a relative risk 3.27 for progression of patients with 11q LOH (95% confidence interval, 1.30–8.18). It is notable that none of the 14 tumors with Myc-N amplification in this study demonstrated LOH at 19q13. A larger prospective study will be necessary to further define the significance of these findings; however, if confirmed, 19q13 LOH in conjunction with DNA index may help identify patients at risk of dying from a recalcitrant LR NB.
Among stage 4 patients, only eight had LOH at 19q13, and four of the eight had large bulky 4N disease. It is of interest that stage 4N NB does not metastasize to bone or bone marrow like classic stage 4 disease and in this way is more similar to bulky LR NB. Six of the eight (including the four stage 4N) stage 4 patients with 19q13 LOH had tumor recurrence at or near the primary site. This rate of local primary recurrence (56%) was high compared with the reported rate of recurrence for other patients (16%) treated with N6 and N7 therapy during the same time period (9). Four patients treated with N7 therapy are alive and well, progression free. The other four treated with less intense regimens died.

Clonal 19q13 LOH in NB Can Be Acquired and Is Closely Associated with Local Recurrence. Nineteen (16%) of the 116 patients relapsed in the primary site after complete or very good partial responses to initial therapy, including 13 (28%) of the 45 LR and 6 (10%) of the 61 stage 4 cases. 19q13 LOH was found in 12 (63%) of the 19 relapsed cases.

Eight of the 17 cases with 19q13 LOH were found in the original diagnostic tumor biopsy. For these, the deletion was detected in all available subsequent specimens, regardless of cytotoxic therapy. In the other 9 cases, 19q13 LOH was detected either in relapse or in postchemotherapy specimens, and the diagnostic biopsy was not available for review. In 2 of these cases, the deletion was shown to be acquired (or selected) during progression because a prior tumor sample retained heterozygosity (Fig. 4).

In 2 cases where the deletion was found in only one of multiple samples from different sites, the relapse was at the site of the tumor in which 19q13 LOH was detected (Fig. 4A). In one case, the deleted region increased in size with subsequent samples and in a cell line derived from the tumor (Fig. 4B). These results suggest that NB clones with 19q13 LOH have a propensity to regrow after surgical debulking and may be a marker of local aggressiveness.

DISCUSSION
Loss of chromosome 19q13 is an uncommon finding in human tumors and has been most frequently reported in ovarian cancer (10) and gliomas (11). Interestingly, 1p36 and 19q13 LOH have demonstrated exquisite specificity for the oligodendroglial lineage among human gliomas and constitute early oncogenic events for these tumors (12, 13). Both NB and oligodendroglial tumors are believed to arise from neuroectodermal precursor cells (14), and both tumor types are characterized by a high incidence of 1p and a low incidence of p53 mutations (11, 15, 16). However, the clinical and biological significance of 19q13 LOH is quite different for these two tumors. Although the deletion represents an early event in oligodendroglioma tumorigenesis and confers good prognosis related to chemosensitivity, 19q13 can be acquired or selected during NB progression and is associated with a high risk for local treatment failure and poor prognosis.

The frequent LOH at 19q in these tumors suggests the location of a tumor suppressor gene; however, none have yet been confirmed. The shortest common region of loss in oligo-
dendroglial tumors has recently been narrowed to a 1.4-Mb region between the markers D19S412 and D19S596 (4), a site overlapping the SRO identified for NB in this study (Fig. 3). Known genes in the region include LIG1 (ligase I, DNA, ATP-dependent), GRLF1 (glucocorticoid receptor DNA binding factor 1), RPL18 (ribosomal protein L18), HNF3G (hepatocyte nuclear factor 3, gamma), and ATP5G1 (ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C, isoform 1). LIG1 is one of four DNA repair/DNA metabolism genes that reside at 19q13.3, the other three (XRCC1, ERCC1, and ERCC2) are outside of the glioma and NB SRO. Other interesting candidate genes, such as the glia maturation factor γ (GMFγ) and the astrocytic NOVA1-like gene or ANOVA, were previously unsuccessfully screened for mutations in glioma tumors (14, 17, 18). More recently, a narrower region has been suggested, extending the 150-Kb SRO between BAC 284K17 and PAC 310F22 contained within the 1.4-Mb region (4, 19). Nine new transcripts were identified in the 150-Kb region including: C5RI, GLTSCR1 (glioma tumor suppressor critical region 1), EHD2, GLTSCR2 (glioma tumor suppressor critical region 2), SEPW1, CRX, STD, cytohesin-2, and synaptogyrin-4. None of these transcribed genes showed mutations or aberrant expression in gliomas with 19q LOH (19). The clinical observation that NB tumors with 19q13 LOH recurred and were resistant to therapy suggested BAX at 19q13 as a candidate gene. Mutation analysis of the BAX gene (11) for 20 NB and 1 NB cell line revealed three different polymorphisms but no mutations. Furthermore, Western blot analysis for cases with 19q LOH failed to show differences in the amount of BAX protein compared with cases without 19q LOH (data not shown).

In this study, we found an association between 19q13.3 and bulky LR tumor without bone or bone marrow metastasis (most commonly clinical stages 3 and 4N), progression despite surgical debulking, and lack of response to standard cytotoxic therapy. LOH can be acquired during the course of the disease and is topographically directly associated with tumor recurrence. This subgroup may be responsible for part of the current controversies regarding conservative management of LR NB (2). There was statistical correlation between 19q13 LOH and outcome for patients with LR NB, suggesting that this marker may prove useful for risk-group classification of NB and assignment of appropriate therapy.

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