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Advances in Brief

Loss of Heterozygosity for Chromosomes 16q and 1p in Wilms' Tumors Predicts an Adverse Outcome[1]

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Abstract

We have prospectively analyzed Wilms' tumors from 232 patients registered on the National Wilms' Tumor Study for loss of heterozygosity (LOH) on chromosomes 11p, 16q, and 1p. These chromosomal aberrations were found in 70 (33%), 35 (17%), and 21 (12%) of the informative cases, respectively. LOH for two of these regions occurred in only 25 cases, and only one tumor harbored LOH at all three sites. There was no statistically significant association between LOH at any of the three regions and either the stage or histological classification of the tumor. Patients with tumor-specific LOH for chromosome 16q had relapse rates 3.3 times higher ($P = 0.01$) and mortality rates 12 times higher ($P < 0.01$) than patients without LOH for chromosome 16q. These differences remained when adjusted for histology or for stage. Patients with LOH for chromosome 1p had relapse and mortality rates three times higher than those for patients without LOH for chromosome 1p, but these results were not statistically significant. In contrast, LOH for chromosome 11p had no effect on measures of outcome. These molecular markers may serve to further stratify Wilms' tumor patients into biologically favorable and unfavorable subgroups, allowing continued use of the clinical trial mechanism in the study of Wilms' tumor.

Introduction

A major objective of the NWTS[3] has been to evaluate increased or intensified therapy for patients at greater risk for relapse because of tumors of higher stage or unfavorable histology, while decreasing the number of chemotherapeutic agents and duration of therapy as well as eliminating radiotherapy for patients with lower stage tumors of favorable histology (1). With current therapy, patients with clinical stage I, II, or III disease have at least an 85% 2-year RFS rate, while those with stage IV disease have an approximately 75% 2-year RFS (1). Although the histological finding of diffuse anaplasia identifies a subgroup of children with only 55% RFS at 2 years, this group accounts for only 5% of all cases of Wilms' tumor (1). Continuation of this refinement of therapy, based on risk of relapse, will require the identification of new prognostic factors. Approximately 30% of Wilms' tumors have been found to harbor LOH for chromosome 11p markers (2, 3), and we have previously reported that 20% of tumors have undergone LOH for polymorphic markers on chromosome 16q (4). In the latter report, one of eight cases also had LOH at a locus mapping to distal chromosome 1p, while LOH for all other chromosomal arms, excluding chromosomes 11p, 16q, and 1p, occurred in less than 5% of informative tumors (4). The possibility that such tumor-specific genetic alterations might correlate with outcome has not been explored previously in Wilms' tumor patients, although in another embryonal tumor, neuroblastoma, loss of either chromosome 1p or 14q has been shown to be independent adverse prognostic factors (5). We have, therefore, prospectively characterized Wilms' tumors from 232 patients for LOH on chromosomes 11p, 16q, and 1p and compared RFS and overall survival between the molecularly defined subgroups.

Materials and Methods

Subjects. Patients were accrued through the mechanism of the Pediatric Oncology Group Study 9046, "A Molecular Genetic Analysis of Wilms' Tumor." This is a prospective study which includes patients less than age 17 years with any renal tumor. Although not a requirement of this biological study, over 95% of the patients were independently registered on the third or fourth National Wilms' Tumor Study, thus making central pathology review and follow-up data available. Importantly, all patients were treated on uniform protocols defined by stage and histology (1). The present analysis included only patients with intrarenal Wilms' tumors of favorable or anaplastic histology (6) for whom both the date of diagnosis and at least one follow-up report had been received. Other histological variants such as clear cell sarcoma, rhabdoid tumor, mesoblastic nephroma, nephroblastomatosis, and extrarenal Wilms' tumors were excluded from the analysis.

DNA Analysis. DNA was extracted from frozen peripheral blood and tumor tissue, restricted with the appropriate enzymes, separated in 0.8–1.2% agarose gels, and Southern blotted using standard methodology. Blots were hybridized in 10% polyethylene glycol, 7% sodium dodecyl sulfate, and 1 M sodium chloride overnight at 65°C and then washed under high stringency conditions. The DNA probes, locus name, restriction enzyme used, and genomic location, respectively, were: chromosome 11p—pTBB2, *HRAS1*, *Taq*I, 11p15.5; pHins310, *INS*, *Pvu*II, 11p15.5; pFSH0.5, *FSH*, *Hind*III, 11p13; p32-1, *D11S16*, *Msp*I, 11p13; chromosome 16q—p79-2-23, *D16S7*, *Taq*1, 16q24; pEKX355, *CTRB*, *Pvu*II, 16q23; pH2α, *HP*, *Eco*RI or *Hind*III, 16q22; chromosome 1p—p1-79, *D1Z2*, *Taq*I, 1p36 (7). A dinucleotide repeat polymorphism in *D16S260* was also analysed in some cases using polymerase chain reaction as described (8).

For chromosome 11p, tumors were classified as having LOH if loss was seen at any informative locus and noninformative only if the patient was constitutionally homozygous at all four loci examined. For the purposes of this analysis, no distinction was made between the 11p13 and 11p15 regions. For chromosome 16q, all cases were initially analyzed at the *D16S7* and *HP* loci. Cases noninformative at *D16S7* (16q24) were analyzed at the adjacent *CTRB* locus (16q23), while patients homozygous at *HP* (16q22) were also tested at *D16S260*, located just centromerically. Tumors with loss at any 16q locus were classified as LOH, and only cases homozygous at all four loci were considered noninformative. On chromosome 1p, only one locus, *D1Z2*, was analyzed. Hybridization with the probe p1-79 yielded a highly polymorphic pattern of multiple bands, and all patients were considered informative.

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[3] The abbreviations used are: NWTS, National Wilms' Tumor Study; RFS, relapse-free survival; LOH, loss of heterozygosity; OS, overall survival.

Table 1 *Distribution of stage and histology by chromosomal LOH*[a]

Chromosomal region	Stage						Histology[c]		
	I	II	III	IV	V	H[b]	FH	UH	UNK
11p									
LOH	20 (33)[d]	16 (42)	14 (29)	11 (38)	8 (28)	1 (33)	65 (35)	2 (13)	3 (38)
No LOH	41	22	35	18	21	2	120	14	5
NI[e]	7	5	6	2	3	0	21	1	1
16q									
LOH	6 (10)	10 (25)	8 (17)	7 (27)	3 (11)	1 (50)	29 (16)	5 (33)	1 (13)
No LOH	55	30	40	19	24	1	152	10	7
NI	7	3	7	5	5	1	25	2	1
1p									
LOH	4 (8)	3 (9)	9 (20)	4 (18)	1 (4)	0 (0)	16 (10)	3 (21)	2 (25)
No LOH	46	31	35	18	23	1	137	11	6
NI	18	9	11	9	8	2	53	3	1

[a] LOH, loss of heterozygosity.
[b] H, tumor arising in a horseshoe kidney.
[c] FH, favorable histology; UH, anaplastic histology; UNK, unknown.
[d] Number of cases with percentage of informative cases in parentheses.
[e] NI, not informative or not done.

Patients with bilateral tumors, when both were analysed, were coded as LOH if either tumor displayed loss of the chromosomal segment.

Statistical Analysis. The two end points were RFS and OS. Patients with persistent disease at last follow up were not coded as failures unless they had evidence of progressive disease. One patient, who died of toxicity without prior relapse, was treated as censored at time of death in the RFS analysis. RFS and OS curves were compared using the log-rank test (9). Relative risks were calculated using the Cox proportional hazards model with and without adjustment for stage or anaplastic histology (10).

Results and Discussion

Of 232 cases which met the eligibility criteria, 17 had tumors with either focal or diffuse anaplasia (unfavorable histology); in 9, the histology was unknown, and the remaining 206 had tumors with favorable histology. Sixty-eight (29%) cases had stage I disease, 43 (19%) stage II, 55 (24%) stage III, 31 (13%) stage IV, 32 (14%) stage V (bilateral tumors), and 3 (1%) had tumors arising in a horseshoe kidney. This distribution is comparable to that seen in the NWTS (1), with the exception of a slight excess of bilateral cases, and therefore suggests that the 232 study cases are not a selected population.

LOH for each chromosomal region was seen in tumors of all stages as shown in Table 1. The proportion of cases with LOH for chromosomes 11p, 16q, or 1p within each stage was not statistically significantly different when analyzed using standard χ^2 tests for homogeneity and trend. Similarly, no significant association between histology and LOH was seen for any of the three chromosomes.

As shown in Table 2, 33% of informative cases harbored tumor-specific LOH for chromosomal 11p markers, 17% for 16q, and 12% for 1p. Only 25 cases had LOH for two of the chromosomal regions, and just 1 of 151 cases analysed and informative for all three chromosomes had LOH at all three sites. Thus, LOH for chromosomes 11p and 16q are largely independent events identifying relatively distinct subsets of patients. There was a possible association between LOH on chromosome 1p and either 11p or 16q with odds ratios of 2.5 (P = 0.10 using a two-sided Fisher exact test).

Analyses of outcome using a log-rank test for the 204 patients whose constitutional DNA was informative for at least one locus on chromosome 16q revealed that those whose tumors had LOH had a statistically significantly worse RFS (P = 0.01) and OS (P = <0.0001) (Fig. 1). Although there was no apparent association between LOH and either stage or histology, the analyses were repeated using the Cox proportional hazards model to account for these previously known prognostic variables. The difference in RFS remained significant when adjusted for histology (P = 0.01) or for stage (P = .03), while for OS these values were P = 0.0002 and P = 0.0001, respectively. Caution is needed in interpreting P for survival since they are based on asymptotic theory, whereas relatively few deaths were observed, 3 (*versus* 7.6 expected) for patients without LOH and 6 (*versus* 1.4 expected) for patients with LOH at 16q. With a median follow-up duration in the LOH and non-LOH groups of only 1.3 and 1.4 years, respectively, the 95% confidence intervals for estimates of 2-year RFS were too wide to be of practical use. Using the Cox proportional hazards model, there was a relative risk of 3.3 for relapse and 11.6 for death for patients with 16q LOH *versus* no LOH.

There were 175 cases available for analysis on chromosome 1p since DNA was no longer available for some of the earlier patients. The estimated relative risk of relapse was 2.7 and of death, 3.2, for

Table 2 *Incidence of LOH by chromosome*

Chromosomal region	LOH	No LOH	NI	% LOH
11p	70	139	23	33
16q	35	169	28	17
1p	21	154	57	12
11p and 16q	10	173	49	6
11p and 1p	9	148	75	6
16q and 1p	6	162	64	4
11p and 16q and 1p	1	150	81	1

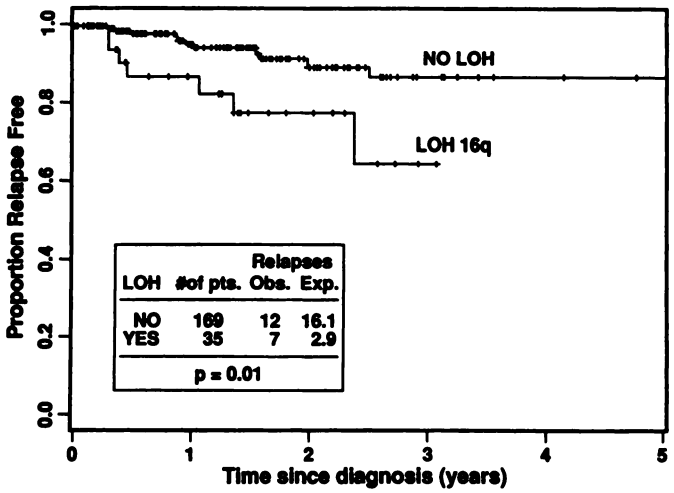

Fig. 1. Relapse-free survival of 204 patients classified by tumor-specific LOH for chromosome 16q.

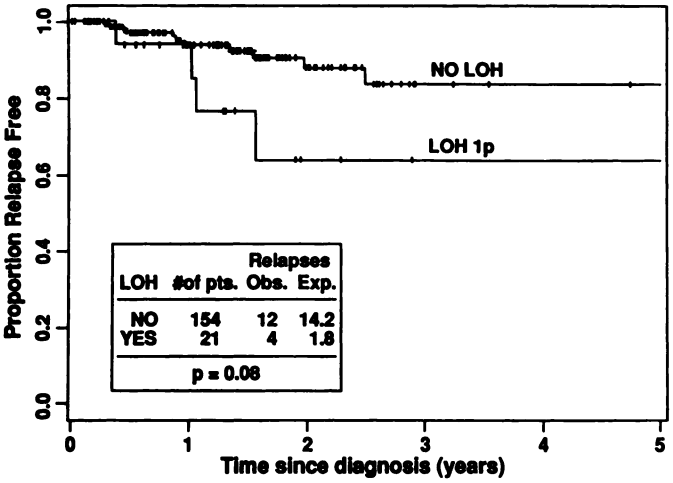

Fig. 2. Relapse-free survival of 175 patients classified by tumor-specific LOH for chromosome 1p.

patients with 1p LOH *versus* no LOH (Fig. 2). However, these differences were not statistically significant ($P = 0.08$ for RFS; $P = 0.14$ for OS). Given the low incidence of the genetic aberration (12%), the overall infrequency of relapse and the short median duration of follow up, the sample size was inadequate to establish a true difference in outcome.

On the other hand, when the cases were classified by LOH on chromosome 11, there was no suggestion of a difference in RFS ($P = 0.74$) or OS ($P = 0.74$). This suggests that the correlations between LOH on chromosomes 16q and 1p and outcome are chromosomal region specific. The fact that so few tumors displayed LOH at multiple sites further implies that LOH is not simply a marker of global genetic instability but more likely infers the location of genes involved in the genesis or progression of the tumor. Thus, although an increased DNA index and complex chromosomal rearrangements have been associated with the anaplastic histology (11) and therefore with adverse outcome, the above data would suggest that 16q and possibly 1p LOH are gene-specific events.

The significant association of 16q LOH with an adverse prognosis suggests that the underlying genetic locus may be involved with tumor progression rather than initiation and that the genetic event may take place in an already established tumor, resulting in further growth advantage to the tumor cells or an increased ability to metastasize. This would be consistent with the facts that no association has been reported between constitutional 16q deletion and the development of Wilms' tumor and that linkage analyses of Wilms' tumor families have excluded this region of chromosome 16 as the location of the inherited predisposition (12). Furthermore, we have noted that significant reduction in one allele, rather than complete loss, occurs more commonly for chromosome 16q loci than for markers on chromosome 11p (data not shown). In some cases, such allelic reduction at *D16S7* has been seen on the same blot as complete allele loss for *HRAS* (chromosome 11p15), excluding contamination of tumor with normal tissue as a viable explanation for this finding. We interpret this allelic reduction as representing LOH in only a subset of tumor cells consistent with a progression event in an already established tumor.

Tumor recurrences were both local and metastatic (data not shown). There were too few instances to ascertain any possible correlations between the site of chromosomal loss and the location of recurrence, therefore, it is not possible to make inferences about the function of the putative underlying tumor genes. The lack of association between LOH for any chromosome and stage, however, would suggest that local invasiveness is not primarily determined by these loci.

16q LOH, which occurs in a number of different carcinomas, has also been found to be associated with an adverse prognosis in hepatocellular carcinoma (13). It is intriguing that hepatocellular carcinoma has been reported as a second malignant neoplasm in survivors of Wilms' tumor (14). Because these were rare events, however, and because they all occurred in patients who received both chemotherapy and radiotherapy, it is possible that these secondary tumors were simply related to the initial therapy.

It is not possible to conduct additional randomized therapeutic trials in favorable histology Wilms' tumor patients without the identification of new prognostic factors because it would require an unrealistic number of patients to statistically prove an increase in survival over 90%. However, if it became possible to identify those 10–15% of lower stage and 25% of higher stage patients destined to relapse, trials could be formulated using alternate therapy for patients with unfavorable molecular findings with further diminution of current therapy for those with favorable patterns.

The current trial will continue both to accrue additional patients and to obtain further follow up. Future analyses may therefore be able to discriminate the prognostic importance of these findings within stage and histological categories.

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