Consistent Association of 1p Loss of Heterozygosity with Pheochromocytomas from Patients with Multiple Endocrine Neoplasia Type 2 Syndromes

Jeffrey F. Moley, Michele B. Brother, Chin-To Fong, Peter S. White, Stephen B. Baylin, Barry Nelkin, Samuel A. Wells, and Garrett M. Brodeur

Departments of Surgery [J. F. M., M. B. B., S. A. W.], and Pediatrics [C.-T. F., P. S. W., G. M. B.], Washington University School of Medicine, St. Louis, Missouri 63110, and the Department of Oncology [S. B. B., B. N.], Johns Hopkins Oncology Center, Baltimore, Maryland 21231

ABSTRACT

Pheochromocytomas and medullary thyroid cancers (MTCs) are neuroendocrine tumors which arise sporadically or as part of the multiple endocrine neoplasia type 2 (MEN-2) hereditary syndromes. The most consistent molecular genetic abnormality which has been described in these tumors is loss of heterozygosity (LOH) of the short arm of chromosome 1 (1p). This finding is particularly interesting because the predisposition gene for the hereditary form of these tumors has been mapped to chromosome 10, but LOH on chromosome 10 in MEN-2 tumors is found rarely. We have used a battery of 1p DNA probes to elucidate the region of loss of 1p in 18 pheochromocytomas and 27 MTCs. Using restriction fragment length polymorphism analysis, we identified loss of all or a portion of 1p in 12 of 18 pheochromocytomas. 1p LOH was identified in nine of nine pheochromocytomas in MEN-2A and -2B patients, compared with only two of seven sporadic pheochromocytomas. We also found 1p LOH in one of two von Hippel-Lindau patients. LOH on 1p was noted in only three of 24 informative MTCs, and these were from patients with MEN-2A. In most of the pheochromocytomas, the entire short arm of chromosome 1p appears to have been lost; however, in three of the non-MEN pheochromocytomas and in three MEN-2A MTCs, the region of loss is smaller, allowing estimation of the smallest region of overlap. The combined data for MTCs and pheochromocytomas suggest that the smallest region of overlap of LOH is bounded by D1S15 (1pter-p22) and D1Z2 (1p36.3), excluding a region around MYCL (1p32). Although other regions of 1p should not be completely ruled out, the data suggest that this region may harbor a tumor suppressor gene or genes whose inactivation is important in the development of these tumors. Furthermore, the strong association between 1p LOH and the MEN-2 syndromes, especially in pheochromocytomas, suggests a relationship between the predisposition gene on chromosome 10 and the loss of the suppressor gene on 1p. Alternatively, other loci may be more important in sporadic disease.

INTRODUCTION

Pheochromocytomas and MTCs are neuroendocrine tumors which originate from adrenal chromaffin cells and thyroid C-cells, respectively (1–4). These tumors occur in both sporadic and familial forms and are heterogeneous in their clinical manifestations. Pheochromocytomas are a common finding in patients with MEN-2 syndromes, but these tumors also occur in patients with other hereditary syndromes, such as neurofibromatosis type 1 and vHL disease. Interestingly, each of these genetic diseases has been mapped to a different chromosome: the MEN-2 syndromes to the pericentromeric region of chromosome 10 (5, 6); neurofibromatosis to 17q11 (7); and vHL syndrome to 3p (8). MTCs can occur in MEN types 2A and 2B, as well as a distinct familial MTC syndrome that maps to chromosome 10 also, but is not associated with other features of the MEN-2 phenotype (9, 10).

The genetic abnormalities underlying these tumors are not well understood. Deletion or LOH in the region of the predisposing locus on chromosome 10 is found rarely in pheochromocytomas and MTCs. Recent reports have noted allelic loss on chromosome 10 in tumors of only 4 of 86 informative cases (11–13). This contrasts with the situation in hereditary retinoblastoma, in which tumors that occur in predisposed individuals achieve homozygosity for a defective RB-1 allele in the majority of cases (14, 15). It is possible that malignant transformation in the MEN-2 syndromes is caused by other mechanisms, such as oncogene activation or the inactivation of a tumor suppressor gene at a different chromosomal locus. Evidence of oncogene activation in these tumors has not been found as yet (16, 17). We (17) and others (18–21) have noted chromosome 1 LOH in hereditary and sporadic pheochromocytomas and medullary thyroid cancers, but extensive mapping of the deleted region has not been previously reported.

In the studies reported here, we used a battery of 15 chromosome 1p probes to analyze 18 pheochromocytomas and 6 chromosome 1p probes to analyze 27 medullary thyroid cancers from patients with sporadic and familial disease to define the locus on 1p that is consistently deleted in these tumors and to determine its relationship to the genetic background of the patient. We found LOH of 1p in all pheochromocytomas from patients with MEN-2A and -2B and in a minority of patients with sporadic pheochromocytomas. LOH of 1p was found in only 3 of the 24 informative MTCs studied, and all occurred in patients with MEN-2A.

MATERIALS AND METHODS

Tumor and Blood Samples. In each case, tumor tissue was obtained in the operating room at the time of therapeutic surgery. The tissue was immediately frozen and stored at −80°C. LCLs were established (22) from 10 ml of blood obtained from the patients at the time of blood drawing. These studies have been reviewed and approved by a human studies internal review committee for Washington University.

DNA Studies. DNA was prepared from tumors and LCLs by standard detergent-proteinase K lysis, followed by organic extraction and extensive dialysis (23, 24). DNA was quantitated by a very sensitive fluorimetric assay (25, 26). Equal amounts of normal genomic and tumor DNA (5 μg) from individual patients were digested with an appropriate restriction enzyme, and DNA fragments were separated by continuous agarose gel electrophoresis. Then, DNA was transferred to a nylon membrane by a modification of the procedure of Southern (27). Filters were baked for 2 h at 80°C, equilibrated in prehybridization solution, and then hybridized overnight with either a plasmid labeled by nick translation (28) or with an isolated DNA fragment labeled by the random hexamer primer (29) technique. Filters were washed and ex-
posed to autoradiography film for 1 to 5 days, and then the film was developed.

Probes. We used 16 DNA probes that identified RFLPs and have been regionally mapped to chromosome 1. These probes include pron-atriodiilatin (Ip36), alkaline phosphatase (Ip36.1–34), a c-src-related protooncogene sequence (c-src-2/c-fgr, or FGR) (Ip36.2–36.1), MYCL (also known as L-myc) (Ip32), N-ras (Ip13), nerve growth factor (bNGF) (Ip13), renin (Ip32 or Ip42) and nine random polymorphic DNA sequences: DIS13 (Ip22-p13); DIS15–16 (Ip1-pter-p22); DIS17 (1pter-p32); DIS18–19 (1pter-p22); DIS21 (1pter-22); DIS57 (1pter-31); and DIZ2 (Ip36.3) (Fig. 2) (30–32).

Analysis of Allelic Loss. Pairs of normal and tumor DNA from each patient were analyzed using probe-enzyme combinations that are known to identify RFLPs in a substantial number of individuals. Normal DNA samples which were polymorphic at a given locus were considered to be informative, and if the tumor DNA demonstrated a loss or marked reduction in the intensity of one of the allelic bands, the tumor was considered to have LOH at that allele. Tumor DNA samples contained varying amounts of normal stromal cells, as confirmed by histology. Therefore, in most cases with LOH at a given allele, there was a faint residual band in the position of the lost allele on the autoradiogram (see Fig. 1). In these cases, we performed densitometry using a computerized gel scanner (Apple Scanner) and densitometry software (courtesy of Dr. Hershel Ginsberg, Amoco Technology). For samples which were informative, results were adjusted for DNA content and background in the following manner. The residual density of the deleted band in the tumor DNA was normalized against the density of the retained band. The band in the constitutional DNA corresponding to the deleted band was normalized against the corresponding retained band, and the ratio of normalized, tumor-band density to the normal retained band. The band in the constitutional DNA corresponding to each probe was measured densitometrically (20). If this value was less than 0.5, indicating a greater than 50% reduction in signal intensity in the tumor DNA band, this was considered to represent LOH.

RESULTS

Representative RFLP studies for three pheochromocytomas (Tumors 2, 8, and 9) are shown in Fig. 1. As can be seen in this figure, individuals with LOH can be identified readily, despite some residual signal from contaminating normal cells in the tumor samples. LOH on Ip was found in all pheochromocytomas from patients with MEN-2A and -2B (see Fig. 2). LOH on Ip was also noted in two of seven patients with sporadic disease, and in one of two patients with vHL syndrome. We have not noted a characteristic pattern of LOH in any specific family or kindred with MEN-2A. In most tumors, the loss was extensive, involving most of the chromosomal arm. In Case 16, however, the region of LOH is bounded proximally by DIS15 (1pter-p22) (30) and distally by DIZ2 (Ip36.3) (31). DIZ2 was also found to flank the deleted region distally in Cases 4 and 19.

In general, there were less tumor tissue and DNA available for the MTCs, because many were detected by clinical screening studies before they were large enough to produce symptoms. Twenty-four of 27 MTCs (14 MEN-2A, 5 MEN-2B, 5 sporadic) studied so far were informative for at least one of six loci (DIZ2, DIS17, DIS57, MYCL, NRAS). Of these, three demonstrated LOH for loci on Ip (Fig. 3). In Case 21, LOH occurred at DIS17 (1pter-p32) (30) and N-ras (Ip13) (30), with retention of heterozygosity at DIZ2 (Ip36.3). In Case 32, LOH occurred at DIS21 (1pter-p22) (30), MYCL (Ip32) (30), and DIS57 (1pter-p31) (32); and in Case 13, LOH was found at DIS17 and DIZ2, but not at MYCL. All of these patients had MEN-2A.

In two individuals, both MTC and pheochromocytoma tissue were examined. These were a patient with MEN-2A (pheochromocytoma Case 9, MTC Case 8) and a patient with MEN-2B (pheochromocytoma Case 11, MTC Case 23) (Figs. 2 and 3). In both patients, LOH was observed in the pheochromocytoma but not in the MTC.

DISCUSSION

Knudson et al. (15) initially postulated a two-mutational model of tumorigenesis to explain the development of hereditary and sporadic types of certain tumors. The applicability of this theory to the MEN-2A and -2B syndromes comes from the fact that tumor development is preceded by characteristic hyperplasia of the thyroid C-cell and adrenal chromaffin cell populations (3, 4) and that MTC and pheochromocytomas develop as multifocal clonal tumors within this cellular background (33, 34). Furthermore, Jackson et al. (35) demonstrated that the average age of onset was 36 yr for hereditary cases of MTC and 52 yr for sporadic MTC.

The MEN-2A and -2B syndromes differ from hereditary retinoblastoma (14) in that the predisposition locus has been mapped to chromosome 10 (5, 6), but deletion or LOH at that locus rarely occurs in the pheochromocytomas and MTCs which develop (11–13). A small deletion, rearrangement, or mutation of the normal allele cannot be completely ruled out; however, these data suggest that malignant transformation in MEN-2A patients is probably caused by some mechanism other than homozygous loss or mutation of the predisposition locus. We have demonstrated a high frequency of LOH for Ip in pheochromocytomas from patients with both hereditary and sporadic disease. This consistent abnormality probably indicates the presence of a suppressor gene which is lost or inactivated in these tumors. The finding of frequent LOH at chro-
We have found that the most consistently deleted region in pheochromocytomas is bounded by the D1Z2 locus (1p36.3) distally and proximally by D1S15 (1pter-p22). This region contains several known oncogenes and other structural genes. Unfortunately, none of these appear to be attractive candidates for the suppressor gene that presumably is the target of this genetic rearrangement. Furthermore, no evidence of rearrangement or amplification was found for any of the probes studied in normal or tumor tissues, including those tumors with LOH (data not shown). Although the region that is consistently lost in our tumors potentially overlaps the 1p36 locus that is consistently deleted in neuroblastomas (39), it extends to a large region of the short arm, suggesting that a more proximal locus may be involved. In the MTCs we studied, one MEN-2A tumor (Case 13) with LOH at D1S17 and D1Z2 demonstrated retention of heterozygosity at MYCL. If this finding is combined with our data on pheochromocytomas, it suggests a potential suppressor locus between MYCL (1p32) and D1S15 (1pter-p22) or between MYCL and D1Z2 (1p36.3). This is consistent with the results of Yang et al. (19) whose data suggest a common region of deletion in pheochromocytomas in the middle of the short arm (1p22 to 1p36). The only known structural gene which has been mapped to the region between MYCL and D1S15 is a human glucose transporter (40). We have not found any evidence of abnormality of this gene at the DNA level (data not shown) in tumors with LOH of 1p nor have we found any abnormality of several structural genes which map to the region between MYCL and D1Z2, including pronatriodilatin, alkaline phosphatase, and a c-src-related protooncogene (FGR).

1p LOH has been demonstrated in other tumors of neuroectodermal origin, such as neuroblastoma (39) and melanoma (41, 42), as well as other tumor types, such as breast cancer (43, 44), colon cancer (45), and liver cancer (46). It is not clear if a single gene or locus is involved in all these cases, or if there are two or more loci on the short arm of chromosome 1 that are involved in different tumor types. There are clear evidence for two distinct suppressor loci on the short arm of chromosome 11 (47-49) and mounting evidence for two loci on the short arm of chromosome 17 (50, 51), so it is reasonable to assume that more than one locus on 1p may be involved in these diverse tumor types. Further investigation should determine if there is a common region on 1p that is involved in tumors of neuroectodermal origin.

Another striking finding was the difference in the frequency of 1p LOH between pheochromocytomas and MTCs. We only found 3 of 27 MTCs with 1p LOH. Even among the 19 patients with MEN-2A or -2B, MTCs showed a low frequency of LOH on 1p (16%). In two individuals, one with MEN-2A and one with MEN-2B, both pheochromocytoma and MTC tissue were examined. LOH was found in the pheochromocytoma tissue but not in the MTCs. This genetic heterogeneity may reflect differences in pathogenesis of these two tumor types, which may be related to clinical presentation, natural history, and histological features of pheochromocytomas and MTCs.

In addition to identifying 1p LOH in the largest series of hereditary tumors reported to date, our studies indicate heterogeneity in the frequency of LOH for 1p in pheochromocytomas, depending on the genetic background of the patient. The frequency of LOH for 1p in pheochromocytomas was 100% (9 of 9) in patients with the MEN-2A and -2B syndromes versus 29% (2 of 7) in patients with sporadic disease (P < 0.001, Fisher's exact test). This difference may reflect alternative molecular mechanisms at work in the development of these tumors. These findings also suggest an interaction between the predisposition locus and the loss of a putative suppressor gene (or genes) on 1p in hereditary pheochromocytomas. Alternatively, other chromosomal loci (such as 3p, 17p, or 22q) may be important in the pathogenesis of sporadic pheochromocytomas.

Another striking finding was the difference in the frequency of 1p LOH between pheochromocytomas and MTCs. We only found 3 of 27 MTCs with 1p LOH. Even among the 19 patients with MEN-2A or -2B, MTCs showed a low frequency of LOH on 1p (16%). In two individuals, one with MEN-2A and one with MEN-2B, both pheochromocytoma and MTC tissue were examined. LOH was found in the pheochromocytoma tissue but not in the MTCs. This genetic heterogeneity may reflect differences in pathogenesis of these two tumor types, which may be related to clinical presentation, natural history, and histological features of pheochromocytomas and MTCs.

1p DELETIONS IN TUMORS OF MEN-2 PATIENTS

![Fig. 2. Analysis for LOH at various chromosome 1 loci in human pheochromocytomas.](image)

LOH FOR CHROMOSOME 1P IN PHEOCHROMOCYTOMAS

![Fig. 3. Analysis for LOH at various chromosome 1 loci in MTCs.](image)

LOH FOR CHROMOSOME 1P IN MEDULLARY THYROID CANCERS

[Image 11x5 to 601x787]
REFERENCES


Consistent Association of 1p Loss of Heterozygosity with Pheochromocytomas from Patients with Multiple Endocrine Neoplasia Type 2 Syndromes

Jeffrey F. Moley, Michele B. Brother, Chin-To Fong, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/4/770

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.