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ABSTRACT

The susceptibility gene for Cowden disease (CD), an autosomal dominant inherited cancer syndrome, has recently been mapped to an approximately 6-cM interval on chromosome subband 10q22-23 between the markers D10S541 and D10S564. CD is characterized by hamartomas of many organ systems, including the thyroid, breast, skin, and gastrointestinal tract, as well as carcinoma of the thyroid and breast. Follicular thyroid adenomas and carcinomas are significant component tumors in CD; thus, we sought to examine their sporadic counterpart tumors for loss of heterozygosity (LOH) of microsatellite markers in the 20-cM region within and flanking the Cowden critical interval. In all, 38 sporadic thyroid tumors were analyzed. LOH within the CD interval was observed in 5 of 19 (26%) follicular thyroid adenomas and 1 of 9 (11%) Hurthle cell adenomas. Furthermore, of these adenomas with LOH, 3 of 4 (75%) were atypical follicular adenomas, whereas 2 of 15 (13%) were typical follicular adenomas. Surprisingly, no LOH was detected in this region in 10 follicular carcinomas. Taken together, it may instead be evidence against a stepwise progression from atypical adenomas to carcinoma. CD, thus, we sought to examine their sporadic counterpart tumors for loss of heterozygosity within the interval defined by D10S1733 and D10S1739. If the LOH observed in these sporadic tumors is related to the CD gene, then the Cowden critical interval can be revised to lie within the interval defined by D10S579 and D10S564. LOH in this narrow interval implicates the CD gene and the flanking regions as important regulator of development in all three germ layers; thus, the gene will likely lend clues to the pathogenesis of thyroid carcinoma (2, 4, 5). Follicular adenomas and follicular carcinomas are the predominant histology in CD.

INTRODUCTION

Follicular thyroid tumors are commonly sporadic but are also found in genetic conditions such as the rare inherited cancer syndrome CD. CD, an autosomal dominant trait with variable expression, is characterized by hamartomas involving multiple organ systems derived from all three germ cell layers and a high risk of breast and thyroid cancers (1, 2). Estimates of up to 50% lifetime risk of breast cancer in affected females and up to 10% lifetime risk of thyroid cancer in affected individuals have been cited (1-3). Thyroid gland abnormalities are the most frequently reported noncutaneous lesions in CD, affecting approximately two-thirds of these patients. These abnormalities include the benign conditions of goiter, adenoma, hyperthyroidism, hypothyroidism, thyroiditis, and thyroglossal duct cysts, as well as the malignant condition of thyroid carcinoma (2, 4, 5). Follicular adenomas and follicular carcinomas are the predominant histology in CD.

The putative CD gene has recently been mapped to 10q22-23 (6). Manifestations of CD involve derivatives of endoderm, mesoderm, and ectoderm; thus, the yet-to-be identified susceptibility gene will likely prove to be an important regulator of both development and neoplasia. Despite the apparent rarity of CD, the syndrome and the susceptibility gene will be a good model system for the study of susceptibility to, as well as the etiology and pathogenesis of, neoplastic development: (a) the susceptibility gene will likely prove to be an important regulator of development in all three germ layers; (b) the hallmark of CD is hamartomas; thus, the gene will likely lend clues to the study of growth, overgrowth, and malignant transformation; (c) two CD component tumors (breast and thyroid) have common sporadic counterparts, and the CD susceptibility gene may well play a prominent role in the pathogenesis of these sporadic tumors.

Because follicular thyroid adenomas and carcinomas are major component tumors of CD, it is possible that the CD gene, although being mutated in the germ-line of CD patients, may also be involved in the development of sporadic follicular thyroid tumors as a result of somatic mutation. Gross LOH along 10q has previously been reported in sporadic follicular thyroid adenomas and carcinomas (7). However, markers within the Cowden region have not been examined previously. Therefore, to determine whether the Cowden locus on 10q22-23 plays a pathogenetic role in the formation of sporadic follicular adenomas and carcinomas of the thyroid, we analyzed a series of 38 tumors for LOH at microsatellite markers in the Cowden critical interval.

MATERIALS AND METHODS

Tumor Specimens. In all, 38 sporadic follicular thyroid tumor samples were obtained. Of these 38 samples, 10 were follicular carcinomas, 9 were Hurthle (oxyphil cell) adenomas, and 19 were nonfunctioning follicular adenomas. The histopathological classification was as suggested by the WHO committee (8).

DNA Preparation. Tumor tissue was collected at the time of surgery and snap-frozen. High molecular weight DNA was obtained from the tissue by phenol-chloroform extraction and ethanol precipitation (9). Sections from each of the tumors were histopathologically examined to ensure that the tumor to be analyzed was representative of the sample as a whole. All tumor samples contained greater than 60% tumor cells. The patients' constitutional DNA was obtained either by DNA extraction from blood leukocytes or from normal thyroid tissue using standard procedures (9).

Detection of LOH. The interval containing the CD gene, D10S541 to D10S564, was originally determined to span 5 cM (10). However, recent mapping of this region suggests that this interval in fact spans a distance of 6 cM (11). Nine dinucleotide-repeat microsatellite markers located on 10q in the interval known to contain the CD gene and the flanking regions directly adjacent to it were used: D10S219 (AFM240w7g); D10S551 (AFM240vf10); D10S541 (AFM205xec3); D10S1765 (AFM337x9b); D10S579 (AFM282y1c1); D10S1735 (AFM269xf9); D10S1739 (AFM362yg5); D10S564 (AFM029xh12); and D10S583 (AFM289zh5; Ref. 11). D10S215 (6) was not
DISCUSSION

We found LOH in the region of the putative Cowden locus in over one-fourth of sporadic thyroid follicular adenomas but in none of the sporadic follicular carcinomas. This paradoxical observation is puzzling yet tantalizing. When considering the lack of LOH in the carcinomas, in the context of significant LOH in the adenomas, two opposing hypotheses can be made. The first, and perhaps more simple, of these hypotheses is that if the Cowden gene is involved in the pathogenesis of these sporadic follicular adenomas, LOH in this region is indirect evidence that it is functioning as a tumor suppressor gene, which, when inactive or dysfunctional, could result in cellular proliferation. The high frequency of LOH in atypical as compared to typical adenomas in this study seems to lend credence to this postulate, if one assumes that atypical adenomas frequently progress to carcinomas. With the currently available data, it is unclear whether thyroid follicular carcinoma genesis progresses from adenoma to atypical adenoma to carcinoma or whether each of these entities is unrelated. The significant 10q22-23 LOH observed in these thyroid adenomas may suggest a combination of hypotheses: that progression of adenoma to atypical adenoma may be related to the Cowden locus in this region but that stepwise progression from atypical adenomas to carcinomas does not occur, i.e., atypical adenomas might be a genetically distinct entity from follicular carcinoma. However, studies of different genetic alterations present in both thyroid adenomas and carcinomas, specifically activating point mutations of the ras proto-oncogene (12, 13) and either LOH or translocation at 3p (14), would argue that thyroid adenomas can in fact progress to thyroid carcinomas. An alternative explanation for the observation of the present study would be that only point mutations occur in the putative Cowden gene in those sporadic adenomas destined for progression to carcinomas. This explanation cannot be excluded at present.

A second hypothesis is that the Cowden locus is an oncogene or

RESULTS

DNA from 38 thyroid tumors was screened for LOH using 9 microsatellite markers between and including D10S219 and D10S583, a 20-cM interval. All tumors were informative for at least three markers between and including D10S541 and D10S564. LOH was not detected at any of these loci in 10 follicular carcinomas (Fig. 1). LOH between the markers D10S541 and D10S564, representing the Cowden critical interval, was identified in 1 of 9 (11%) Hürthle cell adenomas and 5 of 19 (26%) follicular adenomas (Fig. 1). Interestingly, 3 of 4 (75%) atypical follicular adenomas had LOH within the Cowden interval compared to 2 of 15 (13%) typical follicular adenomas. Of note, sample 13 (the Hürthle cell adenoma) was shown to have LOH only at D10S1735 and D10S1739. Flanking markers D10S579 and D10S564 remained heterozygous. In sample 20, LOH was observed at D10S1765 and D10S579 and might extend to include D10S1735 and D10S1739 as well. Unfortunately, the latter two markers were homozygous (i.e., noninformative) in this sample. Inspection of the overall pattern of LOH in the Cowden critical interval demonstrated that the SRO included the markers D10S1735 and D10S1739 (Fig. 1).

Whereas one deletion unit was observed within the Cowden critical interval, it seemed that there were two additional deletion units: (a) one unit involving at least the marker D10S551 and the regions centromeric to it (e.g., samples 13 and 16); and (b) the second unit involving the marker D10S583 (e.g., samples 27 and 30; Fig. 1). LOH in these regions is consistent with those reported previously by Zedenius et al. (7).

Fig. 1. LOH analysis of markers within 10q22-23 in 38 thyroid tumors. 1–10, follicular carcinomas; 11–19, Hürthle cell adenomas; 20–38, follicular adenomas. O, retention of heterozygosity; hatched circles, homozygosity (not informative); •, LOH. SRO, the SRO of markers involved in LOH. Chromosome 10 marker position has been determined by the arbitrary assignment of position 0 for the marker D10S219 and subsequent I-unit increments to represent 1 cM as described in the genetic map published by Généthon (11). *, the CD gene has previously been mapped to this region (see text).
encodes a growth promoter. If this alternative hypothesis were true, adenoma formation would be independent of the Cowden locus, but loss of the Cowden locus would prevent carcinoma formation, thus implicating this locus as an oncogene. This might explain the observations that no LOH in the Cowden region is seen in the sporadic follicular carcinomas, that LOH in this region is observed in a significant proportion of sporadic follicular adenomas (this study), and that no LOH has been observed in one breast carcinoma from a Cowden patient who belongs to a 10q-linked family (15). The overall implication of this final hypothesis is that in CD, germ-line point mutations in the Cowden gene result in gain of function. Which of these two opposing hypotheses is true is currently unknown, and further elucidation awaits isolation of the susceptibility gene.

LOH studies performed on DNA from tumors of sporadic origin have successfully been undertaken to facilitate the mapping of a number of tumor suppressor genes, including the recently identified BRCA2 (16–18). The SRO obtained in this study encompasses D10S1735 and D10S1739. If this represents involvement of the Cowden locus in these sporadic tumors, then the Cowden critical interval is between D10S564 and D10S579, a distance under 5 cM. Known genes within this interval, encoding glutamate dehydrogenase and aorta-specific actin 2, are likely poor candidates due to the function of their gene products in tissue unaffected in CD (19, 20). Two IFN-responsive genes, IFI-56K and IFI-54K (21), are better candidates, although excellent alternatives such as the Kox30 zinc finger gene ZNF33 (22) and Hox11 (23) might be telomeric to this region.

This LOH study has also helped to order the markers in the Cowden critical interval. The two markers D10S541 and D10S1765 have previously been assigned an equivalent genetic position and are located on a single yeast artificial chromosome (11). Similarly, D10S1735 and D10S579 have not been ordered and share an equivalent genetic position (11). The results reported in this study are now able to clearly order these markers based on contiguous stretches of LOH interrupted by definite regions of retained heterozygosity (Fig. 1). D10S541 would seem to be centromeric of D10S1765 based on tumors 20 and 36, which show, in all likelihood, contiguous regions of LOH telomeric to D10S541 because D10S541 remained heterozygous in both tumors. D10S579 would seem to be centromeric of D10S1735 based on tumor 13, which had a contiguous region of LOH telomeric to but not including D10S579 because there is retention of heterozygosity at D10S579. Thus, the order of markers would be as follows: centromere; D10S541; D10S1765; D10S579; D10S1735; D10S1739; D10S564; and telomere (Fig. 1).

It is interesting to observe that a number of genetic abnormalities involving chromosome 10 have now been implicated in thyroid neoplasia. Activating missense mutations of the RET proto-oncogene, mapped to 10q11.2, have been implicated in the development of medullary thyroid carcinoma, both within the familial cancer syndrome multiple endocrine neoplasia type 2 and in sporadic medullary thyroid carcinoma. RET has also been implicated in the development of sporadic papillary thyroid carcinoma by rearrangements that fuse the tyrosine kinase domain of RET with unrelated sequence, leading to the activation of RET in these neoplasms (14). The current study suggests that a second gene on the long arm of chromosome 10, most likely the CD susceptibility locus, is involved in the genesis of sporadic follicular tumours.

Other genes implicated in the genesis of thyroid tumors include the adenomatous polyposis coli gene (APC), albeit at a very low frequency (24), and the p53 gene in anaplastic carcinomas (13). The presence of thyroid neoplasms in patients with adenomatous polyposis coli is not uncommon; however, in Li-Fraumeni syndrome (associated with p53 germ-line mutation), thyroid neoplasms do not occur. Mutations of the tumor suppressor gene p53 are present in many different sporadic tumors (25). p53 has a role in many pathways including apoptosis, DNA replication, DNA repair, and development; thus, the mutation of p53 in sporadic thyroid tumors should be considered unusual.

In summary, we have found a distinct and small deletion unit within the Cowden critical interval at 10q22-23 that is found in sporadic follicular tumors. The surprising observation of LOH in sporadic adenomas, especially atypical ones, but not in carcinomas generates interesting and opposing hypotheses. The gene(s) within the deletion interval, which might represent the putative CD gene, could encode either a growth-promoting or growth-suppressing factor involved in the pathogenesis of both CD and sporadic thyroid tumors. The determination of which hypothesis is correct awaits the isolation and characterization of the susceptibility gene for CD.

ACKNOWLEDGMENTS

We thank Fred Li for helpful discussions and Richard Kolodner, Bruce Ponder, and Jan Vijg for their support. We are grateful to Wendy M. Smith for technical assistance, Krishna Krithivas for assistance with the Genescan software, and the Molecular Biology Core Facility at the Dana-Farber Cancer Institute for running the Genescan gels. Sharice Abdillahi provided administrative support.

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