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Bovée, J.V.M.G.; Zedenius, J.; Wallin, G.; Svensson, A.; Höög, A.; Bäckdahl, M.; Larsson, C.

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ORIGINAL INVESTIGATION

Jan Zedenius · Göran Wallin · Ann Svensson
Judith Bovée · Anders Höög · Martin Bäckdahl
Catharina Larsson

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Abstract Previous studies of follicular thyroid tumors have shown loss of heterozygosity (LOH) on the short arm of chromosome 3 in carcinomas, and on chromosome 10 in atypical adenomas and carcinomas, but not in common adenomas. We studied LOH on these chromosomal arms in 15 follicular thyroid carcinomas, 19 atypical follicular adenomas and 6 anaplastic (undifferentiated) carcinomas. Deletion mapping of chromosome 10 using 15 polymorphic markers showed that 15 (37.5%) of the tumors displayed LOH somewhere along the long arm. Thirteen of these tumors showed deletions involving the telomeric part of chromosome 10q, distal to D10S187. LOH on chromosome 3p was found in 8 (20%) cases. Seven of these also showed LOH on chromosome 10q. In eight cases LOH was seen on chromosome 10q but not 3p. In comparison, the retinoblastoma gene locus at chromosome 13q showed LOH in 22% of the tumors. Most of these also had deletions on chromosome 10q. The results indicate that a region at the telomeric part of 10q may be involved in progression of follicular thyroid tumors.

Introduction

Follicular thyroid tumors serve as a good model for studying possible genetic events in tumor progression. Some steps have been elucidated, and schemes for multi-stage tumorigenesis have been proposed (Fagin 1992; Wynford-Thomas 1993; Farid et al. 1994). A recent study of allelotypes of follicular thyroid tumors has allowed us to suggest the addition of genetic loss of the long arm of chromosome 10 in atypical follicular adenomas and follicular carcinomas to these schemes (Zedenius et al. 1995a).

Follicular thyroid tumors consist of common (trabecular/solid, microfollicular, normofollicular, macrofollicular) adenomas, Hürthle (oxyphil cell) adenomas, atypical adenomas (including oxyphil cell type), and carcinomas (Hedinger et al. 1988). The latter group may be further subdivided into minimally and widely invasive carcinomas, mainly depending on their local growth pattern (Hedinger et al. 1988). Furthermore, there is evidence that differentiated thyroid carcinomas transform to poorly differentiated and anaplastic carcinoma types (Ito et al. 1992; Hadar et al. 1993; van der Laan et al. 1993; Dobashi et al. 1994).

The aim of this study was to investigate the significance of chromosome 10 deletions in follicular tumors of the thyroid gland. The results verify that genetic loss on the long arm may be involved in progression of follicular thyroid tumors.

Materials and methods

Tumor specimens

The study included thyroid tumor samples from 40 patients: 6 anaplastic carcinomas, 15 follicular carcinomas (6 of which were classified as Hürthle carcinomas), and 19 atypical follicular adenomas (5 of which were Hürthle type). The histopathological classification was as suggested by the WHO committee (Hedinger et al. 1988), and is given for each tumor in Table 1.

DNA preparation

High molecular weight DNA was obtained from the snap-frozen tumor tissues by phenol-chloroform extraction and ethanol precipitation according to standard methods. To prove the representativeness of the tumor material, pieces were cut from all specimens for histopathological examination. All tumor samples contained more than 60% tumor cells.

The patients leukocytes (26/40) or normal thyroid tissue (14/40) were used for extraction of constitutional DNA. Thus, the deletion study included paired constitutional and tumor DNA from 40 patients.

J. Zedenius (✉) · G. Wallin · A. Svensson · M. Bäckdahl
Department of Surgery, Karolinska Hospital,
S-171 76 Stockholm, Sweden

J. Zedenius · Judith Bovée · C. Larsson
Department of Molecular Medicine (Endocrine Tumor Unit),
Karolinska Hospital, S-171 76 Stockholm, Sweden

A. Höög
Department of Pathology, Karolinska Hospital,
S-171 76 Stockholm, Sweden

Detection of loss of heterozygosity (LOH)

Two techniques were used for the deletion study: Southern blot hybridization using restriction fragment length polymorphism/variable number of tandem repeats (RFLP/VNTR) markers, and the polymerase chain reaction (PCR)-based detection of microsatellite repeat polymorphisms (i.e., CA repeats).

Three polymorphic markers localized to chromosome 10q were used for Southern hybridization: D10S1/Dry 5–1, D10S4/p1-101, and D10S25/Efd 75 (NIH/CEPH Collaborative Mapping Group 1992). High molecular weight DNA was digested with *Taq*1, separated on agarose gels, and transferred to Zeta-Probe nylon filters (Bio-Rad), which were hybridized and autoradiographed as described (Zedenius et al. 1995a). LOH was detected as either a total absence of signal, or ≥ 50% reduced signal intensity of one of the constitutional alleles in the DNA of the thyroid tumor tissue.

Twelve microsatellite markers located on chromosome 10 were used for the deletion mapping: D10S249/AFM207wd12, D10S211/AFM198wf8, D10S193/AFM095zh7, D10S141, ZNF22, D10S215/AFM205wd12, D10S205/AFM164yd8, D10S187/AFM042xa9, D10S190/AFM065yh11, D10S216/AFM205zd8, D10S217/AFM212xd6, and D10S555/AFM242yc7 (Gyapay et al. 1994; Love et al. 1993a,b). In addition, one marker on chromosome 1pter (D1S243/AFM214yg7), four located on chromosome 3p25-14 (D3S656, D3S1029, D3S1076, D3S1217/MIT-F8), and one on chromosome 13q (Rb 1.20) were analyzed (NIH/CEPH Collaborative Mapping Group 1992, Gyapay et al. 1994; Jones et al. 1992; Yandell and Dryja 1989). All chromosome 10 markers, their relative order and approximate chromosomal locations are given in Fig. 1. PCR reactions were carried out in a final volume of 10 µl, containing 40 ng of high molecular weight DNA, 50 m*M* KCl, 10 m*M* Tris-HCl, pH 8.3, 1.5 m*M* $MgCl_2$, 125 µ*M* of each dNTP, 2 pmol of each oligodeoxynucleotide primer (one of which was end-labeled with ^{32}P), and 0.2 U DNA polymerase (Dynazyme, Finnzyme Oy). The standard thermal cycling conditions were: incubation at 94°C for 4 min, 25 step cycles at 94°C for 1 min, 60°C for 1 min and at 72°C for 1 min with a final extension for 7 min at 72°C. Aliquots of the PCR product were denatured with formamide, heated and electrophoresed on denaturing polyacrylamide gels, which were fixed, dried, and subjected to autoradiography. LOH was detected as described above.

Results

We investigated 40 thyroid tumors for LOH on five chromosomal arms using 21 polymorphic markers. The histopathological diagnoses and a summary of the LOH data for each tumor is given in Table 1. Fifteen of the 40 tumors (37.5%) showed LOH somewhere on the long arm, while 8 (20%) showed LOH on the short arm of chromosome 10. LOH at chromosome 1p was found in 5 of 36 (14%) informative cases. Four markers on chromosome 3p together showed LOH in 8 of the 40 tumors (20%). In 7 of these all informative markers on 3p showed LOH, indicating loss of the whole chromosomal arm. In only 1 case (no. 20), was LOH found at chromosome 3p but not 10q, while in 8 cases, LOH was seen on chromosome 10q but not 3p. LOH of the retinoblastoma gene locus (Rb 1.20) at chromosome 13q was seen in 7 of 32 (22%) informative cases; again few of these (nos. 3 and 37) did not show LOH at chromosome 10.

Fifteen polymorphic markers were used for deletion mapping of chromosome 10 (Figs. 1 and 2). This revealed that six of the tumors seemed to have lost the entire chromosome (nos. 18, 24, 25, 29, 31, and 33). Five of these were atypical adenomas, and one was a minimally inva-

Table 1 Histopathological classification and summary of the loss of heterozygosity (LOH) analysis results of each of the 40 tumors

Tumor no.	Histopathological diagnosis[a]	LOH data[b] for chromosome				
		1p	3p	10p	10q	13q
1	ATC, no diff.	+	+	+	+	+
2	ATC, no diff.	–	+	+	+	+
3	ATC, PTC diff.	+	+	+	+	LOH
4	ATC, PTC diff.	+	+	+	+	+
5	ATC, foll diff.	+	LOH	LOH	LOH	LOH
6	ATC, foll diff.	+	LOH	+	LOH	+
7	FTC, WI	+	LOH	+	LOH	LOH
8	FTC, WI	LOH	+	+	LOH	+
9	FTC, WI	+	+	+	+	+
10	FTC, insular, WI	–	+	+	+	+
11	FTC, MI	+	+	+	+	+
12	FTC, MI	+	–	+	+	NA
13	FTC, MI	+	+	+	+	+
14	FTC, MI	+	+	+	+	+
15	FTC, MI	+	+	+	+	+
16	HTC, WI	+	+	+	+	+
17	HTC, WI	LOH	+	+	+	–
18	HTC, MI	–	LOH	LOH	LOH	NA
19	HTC, MI	+	+	+	LOH	NA
20	HTC, MI	+	LOH	+	+	+
21	HTC, MI	+	+	+	LOH	+
22	AFA	+	+	+	+	+
23	AFA	+	+	+	+	+
24	AFA	+	+	LOH	LOH	+
25	AFA	LOH	LOH	LOH	LOH	–
26	AFA	+	+	+	+	–
27	AFA	+	+	+	+	+
28	AFA	+	+	+	+	+
29	AFA	LOH	LOH	LOH	LOH	LOH
30	AFA	+	+	+	+	+
31	AFA	+	+	LOH	LOH	LOH
32	AFA	+	+	+	+	–
33	AFA	LOH	LOH	LOH	LOH	LOH
34	AFA	+	+	+	+	+
35	AFA	+	+	+	+	+
36	AHA	+	+	+	LOH	+
37	AHA	+	+	+	+	LOH
38	AHA	+	+	LOH	LOH	–
39	AHA	+	+	+	LOH	+
40	AHA	+	+	+	+	+

[a] Histopathological diagnosis according to the classification by the WHO committee (Hedinger et al. 1988). Within each diagnostic group, the patients are listed consecutively. *ATC* anaplastic thyroid carcinoma, *FTC* follicular thyroid carcinoma, *HTC* Hürthle carcinoma, *AFA* atypical follicular thyroid adenoma, *AHA* atypical Hürthle adenoma, *PTC diff* parts within the specimen with papillary thyroid carcinoma differentiation, *foll diff* parts within the specimen with follicular structures, *WI* widely invasive, *MI* minimally invasive

[b] Accumulated results from the LOH analysis. + retained heterozygosity, – not informative, *LOH* loss of one of the constitutional alleles in the tumor DNA (at chromosome 3p and 10 as shown with at least one of the markers used), *NA* not analyzed

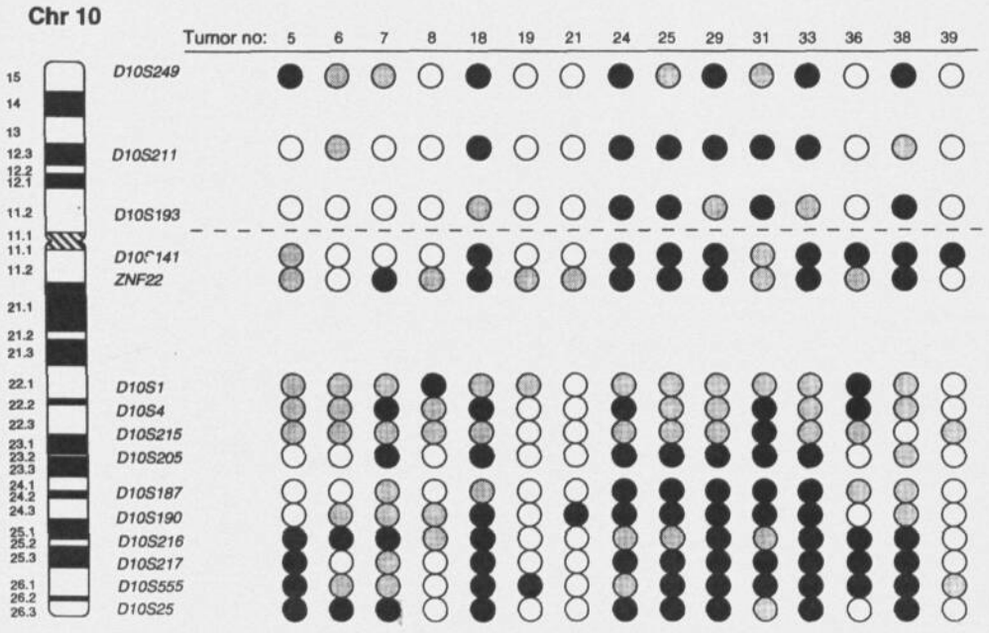

Fig. 1 Tumors in this study showing LOH on chromosome 10. The 15 markers used for the deletion mapping are given to the right of an ideogram of the chromosome. The relative order of the markers is according to the GDB Map C10M50 and the NIH/CEPH Collaborative Mapping Group (1992). The physical location of the markers on the chromosome is approximate. Tumor numbers refer to Table 1. *Empty circles* symbolize retained heterozygosity, *gray circles* homozygous alleles (i.e., marker not informative for the locus), and *black circles* LOH. The *dashed line* indicates the centromere

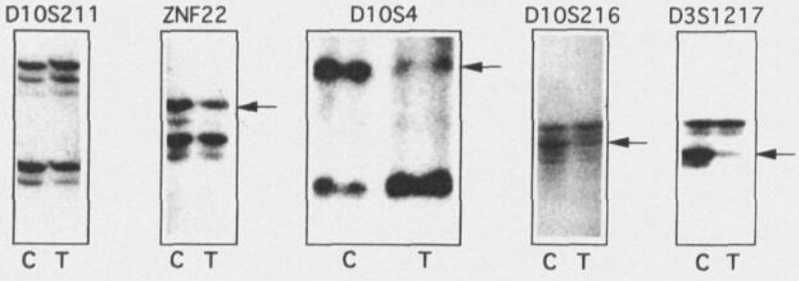

Fig. 2 Autoradiograms showing LOH on chromosomes 10q (markers ZNF22, D10S4 and D10S216) and 3p (marker D3S1217), and retention of heterozygosity on chromosome 10p (marker D10S211) in the follicular thyroid carcinoma from patient number 7. The markers used are given at the top of each autoradiogram, and the lost allele is indicated by an *arrow*. *C* constitutional DNA, *T* tumor DNA

sive Hürthle carcinoma. Thirteen of the 15 tumors with LOH on 10q showed deletions involving the distal part of the chromosomal arm (telomeric of D10S187). However, a single minimal region of overlapping deletions could not be identified; for example, tumor 6 showed retained heterozygosity at D10S217, but losses at both centromeric and telomeric loci. In addition, four tumors (no. 8, 36, 38 and 39) had deletions toward the centromere. Altogether, three putative minimal regions of overlapping deletions may be identified: D10S187-D10S216 and D10S217-D10S25 in the telomeric part, and D10S141-ZNF22 at the centromere.

In contrast to other tumor types (Thibodeau et al. 1993), microsatellite instability was not seen in any tumor.

Discussion

In several tumor types, detection of genetic loss by studying LOH has been a useful method to localize putative tumor suppressor genes and indicate genetic events involved in tumor progression (Zedenius et al. 1995b). Former studies of thyroid tumors have implicated LOH at chromosome 11q13 in follicular adenomas (Matsuo et al. 1991), at chromosome 3p in follicular carcinomas (Herrmann et al. 1991; Roque et al. 1993), and at chromosome 10q in atypical adenomas and carcinomas (Zedenius et al. 1995a). However, the number of carcinomas was small in the latter study. To investigate further the frequency of 3p and 10q deletions in malignant follicular thyroid tumors and their putative impact on tumor progression, we studied LOH in several atypical adenomas, follicular carcinomas, and anaplastic carcinomas.

In the study of Herrmann et al. (1991), all six carcinomas investigated showed deletions of chromosome 3p, mainly involving large parts of the arm. We used four markers at chromosome 3p25-14. LOH was found in 20% of the tumors, but only a single tumor showed LOH at this location and not on chromosome 10q, which is in contrast to earlier reports (Jenkins et al. 1990; Herrmann et al. 1991; Roque et al. 1993). Although small interstitial deletions cannot be excluded, we conclude that LOH on 3p is probably not a prerequisite for development of follicular thyroid carcinomas.

We also investigated chromosome 1p for LOH owing to the fact that in the former allelotype study, two of three carcinomas showed LOH at this location. In the present study, 14% of the tumors showed LOH at 1pter, probably not exceeding the level of "background" LOH.

LOH at the retinoblastoma locus on chromosome 13q was higher, 22%, as compared with the number in our former study (Zedenius et al. 1995a). A recent report has suggested the involvement of this gene in 55% of follicular thyroid carcinomas, but not in adenomas (Zou et al. 1994). The same has been shown for parathyroid tumors, where only carcinomas showed LOH at this locus (Cryns et al. 1994). Our results do not strongly support the involvement of this gene in follicular thyroid tumors, but indicate that a subset of tumors may delete this gene during their development. Notable is the deletion in one of the anaplastic carcinomas (no. 3), which was the only deletion found for this tumor.

Despite the fact that the overall LOH frequency was fairly low in our earlier allelotype study, some LOH patterns were discernible (Zedenius et al. 1995a). The majority of tumors with atypical features (i.e., atypical adenomas and carcinomas), but none of 58 common adenomas, showed LOH on 10q. In the present material, the overall frequency of LOH at 10q was 37.5%. Six tumors, five of which were atypical adenomas, showed LOH on the entire chromosome. In contrast, in follicular carcinomas the deletions were more restricted to 10q (Table 1, Figs. 1 and 2).

In addition, two of the anaplastic carcinomas showed LOH at 10q (nos. 5 and 6). These were the only anaplastic tumors displaying histopathological patterns resembling a follicular differentiation within or close to the undifferentiated tumor. The two anaplastic tumors with papillary carcinoma structure adjacent to the anaplastic carcinoma (nos. 3 and 4) did not show LOH on 10q. It cannot be proven that these four anaplastic tumors emerged as a result of de-differentiation of the differentiated counterparts, but the difference in LOH pattern may favor the hypothesis of a tumor suppressor involved specifically in progression of follicular thyroid tumors.

LOH on the long arm of chromosome 10 has also been found in the progression of other tumor types, e.g., glioblastomas (Leon et al. 1994), malignant meningiomas (Rempel et al. 1993), malignant melanomas (Isshiki et al. 1993), and bladder carcinomas (Wang et al. 1994). In an earlier paper, we touched upon an interesting finding of an aggressive follicular thyroid carcinoma in a young girl with a constitutional ring chromosome, most likely lacking genetic material at the distal part of chromosome 10q (Sparkes et al. 1978; Tommerup and Lothe 1992). In the present study, more than one-third of the tumors showed LOH at chromosome 10q (Fig 1). All but two of these had deletions involving regions distal to D10S187. This comparably frequent finding in tumors with atypical or malignant features indicates a tumor suppressor gene in this region that is involved in progression of follicular thyroid tumors.

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