DNA Copy Number Changes in Thyroid Carcinoma

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The genetic changes leading to thyroid cancer are poorly characterized. We studied DNA copy number changes by comparative genomic hybridization (CGH) in 69 primary thyroid carcinomas. In papillary carcinoma, DNA copy number changes were rare (3 of 26, 12%). The changes were all gains, and they were associated with old age ($P = 0.01$) and the presence of cervical lymph node metastases at presentation ($P = 0.08$). DNA copy number changes were much more frequent in follicular carcinoma (16 of 20, 80%) than in papillary carcinoma ($P < 0.0001$), and follicular carcinomas had more often deletions (13/20 versus 0/26, $P < 0.0001$). Loss of chromosome 22 was common in follicular carcinoma ($n = 7$, 35%), it was more often seen in widely invasive than in minimally invasive follicular carcinoma (54% versus 0%, $P = 0.04$), and it was associated with old age at presentation ($P = 0.01$). In three of the four patients with follicular carcinoma who died of cancer, the tumor had loss of chromosome 22. DNA copy number changes were found in 5 (50%) of the 10 medullary carcinomas studied. Four of these five carcinomas had deletions, and in two of them there was deletion of chromosome 22. Eleven (85%) of the thirteen anaplastic carcinomas investigated had DNA copy number changes, of which five had deletions, and one had deletion of chromosome 22. The most common gains in anaplastic carcinoma were in chromosomes 7p (p22-pter, 31%), 8q (q22-qter, 23%), and 9q (q34-qter, 23%). We conclude that DNA copy number changes are frequent in follicular, medullary, and anaplastic thyroid carcinoma but rare in papillary carcinoma when studied by CGH. Loss of chromosome 22 is particularly common in follicular carcinoma, and it is associated with the widely invasive type. (*Am J Pathol* 1999, 154:1539–1547)

The great majority of all thyroid cancers are either papillary, follicular, or anaplastic carcinomas, which are thought to be derived from follicular cells. Only 5% to 10% are medullary carcinomas, which originate from the

C-cells.¹ This histopathological classification of thyroid carcinomas into four major subtypes has been considered as established, and each of the four entities have typical clinical features. Papillary carcinoma frequently gives rise to cervical lymph node metastases, but distant metastases are rare. This pattern is reversed in follicular carcinoma, which often has distant bone metastases, but cervical lymph node metastases are rare. Papillary and follicular carcinoma are frequently found in the same thyroid as anaplastic carcinoma, suggesting that some anaplastic carcinomas originate from pre-existing differentiated carcinoma.^{2,3}

The molecular genetic events in the evolution of different types of thyroid carcinomas are poorly characterized. However, the central role of mutations in the RET proto-oncogene, located at 10q11.2, in the genesis of hereditary medullary thyroid carcinoma is now well recognized, and used in screening of this disorder. More than 95% of patients with MEN 2A have missense germ line mutations that involve either exon 10 or 11 of this receptor tyrosine kinase,⁴ and all patients with MEN 2B have the same missense mutation in RET exon 16 at amino acid position 918 within what is predicted to be the tyrosine kinase catalytic domain.⁵ Moreover, sporadic medullary carcinomas have somatic mutations in RET codon 918 in 33% to 67% of cases,⁶ and familial non-MEN medullary carcinomas display also frequently RET mutations.⁷ A hypodiploid chromosomal number in the range of 34 to 44 has been found in primary medullary carcinoma tissue.^{8,9} Medullary carcinoma has been found to be associated with a constitutional minute deletion in the short arm of chromosome 20 (del(20)(p12.2)).¹⁰

In cytogenetic studies, only ~30 cases with clonal chromosomal abnormalities have been described in papillary carcinoma.^{11–19} The karyotypic abnormalities are usually simple. An intrachromosomal rearrangement inv(10)(q11q21) resulting in the juxtaposition of sequences encoding the intracellular tyrosine kinase domain of RET with 5' sequences from one of three unrelated genes has been considered as characteristic and has been identified in up to 30% of papillary carcinomas.^{12,14,15,17,20} In many cases, it has been the only anomaly. Activation of other receptor tyrosine kinase genes, such as the proto-oncogenes TRK (encodes a cell

Supported by grants from the Cancer Society of Finland, Academy of Finland, Helsinki University Central Hospital Research Fund, and Clinical Research Institute Helsinki University Central Hospital.

Accepted for publication February 3, 1999.

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surface receptor for nerve growth factor) and MET have also been reported in papillary carcinoma.^{17,21-23}

Cytogenetic information is limited also in follicular carcinoma, and only a few tumors have been examined.^{11-14,24-26} The short arm of chromosome 3 has been reported to contain rearrangements, and a minimal common deleted region at 3p25-pter has been detected.^{14,25} Jenkins et al¹² reported aberrations that were mainly deletions in three follicular carcinomas investigated, and we found a loss of chromosome 22 in 7 of the 13 follicular carcinomas studied.²⁷ Similarly, only few anaplastic carcinomas with an abnormal karyotype have been described,^{12,28} and the molecular mechanisms involved in the genesis of this type of thyroid carcinoma are unknown. Point mutations in the p53 tumor suppressor gene appear to be frequent in anaplastic carcinoma, but not in differentiated thyroid carcinomas.²⁹

This paucity of cytogenetic data on thyroid neoplasms may reflect difficulties in performing karyotype studies on solid tumors, such as finding complex karyotypes and having only a low mitotic index of neoplastic cells in short-term cultures. In the present study we screened genetic imbalances in a series of thyroid carcinomas by comparative genomic hybridization (CGH). This method does not require tumor cell cultures, and it may be performed even from DNA extracted from formalin-fixed and deparaffinized tissue.³⁰ To our knowledge, this is the first study where DNA copy number changes of different types of thyroid carcinomas have been investigated by this method. Papillary and follicular thyroid carcinomas are often grouped together as differentiated thyroid carcinomas, but the present results show that the DNA copy number change patterns are widely different in these two types of thyroid cancer.

Materials and Methods

Patients

The series consists of 69 patients with thyroid carcinoma diagnosed in the Department of Pathology, Helsinki University Central Hospital, between 1981 and 1997. The patients were chosen for analysis based on the availability of fresh-frozen tumor tissue ($n = 53$) or randomly whenever paraffin-embedded tissue was used ($n = 16$). Twenty-six of the carcinomas were of the papillary, twenty follicular, ten medullary, and thirteen of the anaplastic histological type. The histological sections were re-examined and the tumors were reclassified according to the World Health Organization (WHO) classification³¹ by one of us (K. Franssila). The clinical stage was determined according to the International Union Against Cancer (UICC) TNM classification.³² The clinical data are shown in Tables 1 and 2.

DNA Extraction

Histological sections from frozen tissue were cut, stained with toluidine blue, and examined for the presence of representative tumor tissue. In all cases at least 70% of

the cells analyzed were cancer cells. From each tumor specimen 20 to 30 5- μ m sections were cut, and genomic DNA was isolated as described elsewhere.^{30,33} In one case of anaplastic carcinoma (case 8, Table 2) the sample did not contain enough DNA for a CGH analysis, and we multiplied the genomic DNA by using degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR). DNA of peripheral blood lymphocytes obtained from healthy male and female donors was extracted according to standard procedures and was used as a reference in the CGH analyses.

Comparative Genomic Hybridization

CGH was performed according to the method of Kallioniemi et al³⁴ with a modification using fluorochromes conjugated to a mixture of dCTP and dUTP for standard nick translation.³⁵ Tumor DNA was labeled with fluorescein isothiocyanate (FITC)-dUTP and FITC-dCTP (DuPont, Boston, MA), and the normal reference DNA (extracted from the blood of a healthy man or a woman) was labeled with Texas-Red-dUTP and Texas-Red-dCTP (DuPont) in a standard nick-translation reaction. Equal amounts (1 μ g) of the labeled test and reference probes were used for hybridization with 10 μ g of unlabeled human Cot-1 DNA to block the binding of repetitive sequences in 10 μ l of the hybridization buffer (50% formamide, 10% dextran sulfate, 2X SSC (1X SSC is 0.15 mol/L sodium chloride/0.015 mol/L sodium citrate, pH 7)). The DNA was then denatured for 5 minutes at 75°C before applying it to normal lymphocyte preparations. Before hybridization, the metaphase preparations were dehydrated in a series of 70%, 80%, and 100% ethanol concentrations and denatured at 65°C for 2 minutes in a formamide solution (70% formamide/2X SSC). The slides were then dehydrated on ice as described above. They were then treated with proteinase K at 37°C for 7.5 minutes (0.2 μ g/ml in 20 mmol/L Tris/HCl, 2 mmol/L CaCl₂, pH 7) and once again dehydrated in a series of increasing ethanol concentrations as indicated above. Hybridization was performed in a moist chamber at 37°C for 48 hours. Post-hybridization washes were as follows: three times in 50% formamide/2X SSC, pH 7, twice in 2X SSC, and once in 0.1X SSC at 45°C followed by 2X SSC and 0.1 mol/L NaH₂PO₄/0.1 mol/L Na₂HPO₄/0.1% Nonidet P-40, pH 8, and distilled water at room temperature for 10 minutes each. The slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI) at a concentration of 0.1 μ g/ml in an anti-fade solution. To confirm the CGH results, additional hybridization experiments using the reverse-labeling system, ie, tumor DNA labeled with Texas Red and reference DNA with FITC, were performed on some specimens.

Digital Image Analysis

The hybridizations were analyzed using an Olympus fluorescence microscope and an ISIS digital image analysis system (Metasystem, Altusheim, Germany) based on an integrated high-sensitivity monochrome CCD camera

Table 1. Clinical Data and Comparative Genomic Hybridization Findings in 23 Papillary and 20 Follicular Carcinomas of the Thyroid

Case	Age (years)	Sex	TNM	Copy number changes	Follow-up
Papillary carcinoma					
1 to 23	6, 18, 21, 33, 34, 34, 35, 37, 39, 42, 44, 44, 46, 49, 49, 50, 52, 54, 55, 63, 76, 78, 80	F <i>n</i> = 18 M, <i>n</i> = 5	T1N0M0, <i>n</i> = 2 T2N0M0, <i>n</i> = 10 T2N1M0, <i>n</i> = 2 T3N0M0, <i>n</i> = 1 T3N1M0, <i>n</i> = 3 T4N0M0, <i>n</i> = 3 T4N1M0, <i>n</i> = 1 TXNXM0, <i>n</i> = 7	Normal	21 alive, 4-9 years 1 DOD 4 years 1 DOD 7 years
24	78	F	T3N1M0	+5q14-23.3	DOD 5 years
25	81	F	T3N1M0	+5q23.3-qter, +7, +17, +21q22.1-qter	Alive 5 years
26	73	F	T2N0M0	+1q23-qter	Alive 8 years
Follicular carcinoma					
Minimally invasive					
1	34	F	T2N0M0	Normal	Alive 1 year
2	78	F	T3N0M0	Normal	DOD 2 years
3	53	M	T2N0M0	+17q	Alive 4 years
4	57	F	T2N0M1	Normal	Alive 4 years, M+
5	33	M	T3N0M0	+9q11.1-p13	Alive 1 year
6	73	F	T3N0M0	+12q11-q16, +12q22-qter, +19p, +19q13.2-qter, -13q21-q22.1	Alive 1 year
7	66	F	T2N0M1	+17q, -22q12.3-qter	Alive 3 years
Widely invasive					
8	79	M	T1N0M0	+1q22-qter, -1p21-22	Alive 5 years
9	76	M	T4N1M1	-13, -22	Alive 4 years
0	77	F	T1N0M1	-22	DOD 1 year
11	81	F	T3N0M0	+1q22-qter, -1p, -22	DOD 2 years
12	71	F	T3N0M0	Normal	Alive 4 years
13	79	F	T2N0M0	-22	Alive 3 years
14	85	F	T3N0M0	-1p13-23, -21, -22	Alive 1 year
15	68	F	T2N0M0	+1q24-qter, -1p, -9, -13, -18, -22	DOD 10 years
16	68	F	T3N0M0	-13, -22	Alive 6 years, M+
17	79	M	T4N0M0	+17q12-qter, -13q12.3-qter	Alive 1 year
18	74	F	T3N0M0	+1q11-q41	Alive 3 years, M+
19	53	F	TXN0M0	+1, +4, +5, +7, +12, +14, high14q11.2-q22, +18, high18q12.1-q21, -11q14-qter	Alive 1 year
20	66	M	T2N0M0	+5, +X, +7, +12, +18, +19, +20	DOI 4 years

F, female; M, male; DOD, dead of disease; DOI, dead of intercurrent disease; M+, alive with distant metastases.

and automated CGH analysis software. The three-color images with red, green, and blue were acquired from 8 to 10 metaphases. Only metaphases of good quality with strong uniform hybridization were included in the analysis. Chromosomes not suitable for CGH were excluded from the analysis (eg, chromosomes that were heavily bent or overlapping or those that had overlying artifacts). Chromosomal regions were interpreted as over-represented (gained) when the red-to-green ratio exceeded 1.17 and under-represented (lost) when the ratio was less than 0.85 (Figure 1). Ninety-nine percent confidence limits with 1% error probability were used to confirm the interpretation. The cut-off values were taken from negative control experiments by using differentially labeled male DNA *versus* female DNA. A positive control with known chromosomal aberrations and a negative control were included in each hybridization to verify the reliability of the method. Chromosomal regions in the centromeric areas of chromosomes 1, 9, 16, and Y and the p-arms of acrocentric chromosomes were discarded from the analysis because of their large heterochromatic areas.

Degenerate Oligonucleotide Primed PCR

A 5- μ L volume of the extracted DNA was used as a template for DOP-PCR with a universal primer (5'-CCGACTCGAGNNNNNNATGTGG-3', with N = A, C, G, or T) with some modifications.^{36,37} We applied Thermo-sequenase enzyme (Amersham, Cleveland, OH) in 1:10 dilution (3 U/reaction) in a dilution buffer (10 mmol/L Tris/HCl, pH 8.0, 1 mmol/L 2-mercaptoethanol, 0.5% Tween-20, 0.5% Nonidet P-40) in a volume of 10 μ L (26 mmol/L Tris/HCl, pH 9.5, 6.5 mmol/L MgCl₂, 0.2 mmol/L dNTPs, 1 μ mol/L universal primer) applying six cycles of 94°C for 1 minute, 30°C for 3 minutes, 65°C for 5 minutes, and final extension at 72°C for 10 minutes followed by high-stringency cycles consisting of an initial melting at 95°C for 3 minutes and 30 to 35 cycles of 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes, with a final extension of 72°C for 5 minutes, in a volume of 50 μ L using the same reaction conditions as above except 2 μ mol/L primer.

Table 2. Comparative Genomic Hybridization Findings in Medullary and Anaplastic Carcinomas of the Thyroid

Case	Age (years)	Sex	Copy number changes
Medullary carcinoma			
1-5	18,34,34,38,55	F, <i>n</i> = 3 M, <i>n</i> = 2	Normal
6	38	M	+1q21-qter, +6, +7p, +11q, +21q21-qter
7	45	M	-18p, -22
8	38	F	-1p31.1-p32, -3, -13, -18q21-qter
9	48	M	-3q26.2-q29, -13
10	64	M	-22
Anaplastic carcinoma			
1-2	86 77	F F	Normal
3	83	F	+6p12-pter, +5p13-pter
4	74	F	+3p12-p21
5	48	M	+8q21-qter
6	74	M	+9q34-qter, +21q22-qter
7	87	F	+Xp11.2-pter, +7p21-pter
8	78	F	-18p, -22
9	60	M	+2q11.1-q24, +5q34-qter, +8q21.3-qter, +19q13.1-q13.2, +7p22-pter
10	74	M	+9q22.3-qter
11	72	M	+3q, -18q
12	69	M	+X, +7p21-pter
13	64	F	+3q13.3-qter, +5p, +Xq12-qter, +7p13-pter, +8q22-qter, +9, +10q25-qter, +11p11.2-q23.3, +12q21-qter, +15q22-qter, +20q, -4q34-qter, -13

F, female; M, male.

Fluorescence in Situ Hybridization (FISH)

To confirm deletion of chromosome 22, two-color FISH was performed using an LSI DiGeorge/VCFS probe mixture (purchased from Vysis, Ahdiagnostics, Skarholmen, Sweden). The LSI DiGeorge/VCFS probe mixture contains a SpectrumOrange TUPLE 1 probe located at 22q11.2 and a SpectrumGreen LSI ARSA (arylsulfatase A gene) probe that maps close to the telomeric end of 22q at 22q13.3.

Deparaffination of nuclei preparations was done in xylene for 15 minutes at +65°C and three times for 5 minutes each at room temperature, followed by dehydration in a 100%, 85%, 70%, and 50% ethanol series (5 minutes each at room temperature). To allow for penetration of the probe, the slides were treated in 1 mol/L sodium thiocyanate at +70°C for 15 minutes, followed by treatment in 0.05 N HCl at +37°C for 10 minutes and by 5 mg/ml pepsin in 0.05 N HCl at +37°C for 20 minutes. Hybridization was performed according to the standard protocols. The slides were denatured in 70% formamide/2X SSC, pH 7, at +75°C for 5 minutes and counterstained with 125 ng/ml DAPI in an anti-fade solution. From each preparation a minimum of 200 morphologically intact and non-overlapping nuclei were scored using an Olympus fluorescence microscope (Olympus, Tokyo, Japan). Normal lymphocytes were used as controls, and two hybridization signals for the LSI DiGeorge/VCFS probes at the expected locations were found in ~90% of the interphase lymphocyte nuclei. Chromosome 22 was considered to be deleted if only one signal was detected in >20% of cells.

Statistical Analysis

Frequency tables were analyzed with Fisher's exact test, and non-normal distributions between groups were compared with Mann-Whitney's test. All *P* values are two tailed.

Results

Papillary and Follicular Carcinomas

DNA copy number changes were rare in papillary carcinoma; they were found in only 3 (12%) of the 26 tumors investigated. All changes were gains and were found in five different chromosomes (Figure 2, top). In one case a gain of two whole chromosomes (chromosomes 7 and 17) was detected (case 25, Table 1). Gains were found also in chromosomes 1q23-qter, 5q14-23.3, 5q23.3-qter, and 21q22.1-qter. The patients with DNA copy number changes were older (all older than 70) than those without changes (*P* = 0.01). Patients with cancer with DNA copy number changes tended to have more often cervical nodal metastases than those with a normal CGH profile (*P* = 0.08).

DNA copy number changes were much more common in follicular carcinoma than in papillary carcinoma (*P* < 0.0001). As many as 16 (80%) of the 20 follicular carcinomas studied had DNA copy number changes (Figure 2, bottom). The total number of changes detected was 48, and the median number of changes found was 2 (range, 0 to 8). Whereas in papillary carcinoma no dele-

Figure 1. Mean red-to-green ratio profiles of selected chromosomes reflecting DNA sequence copy number changes in thyroid carcinoma. Profiles are those of chromosomes 7 (anaplastic carcinoma), 13 (medullary carcinoma), and 22 (follicular carcinoma), which showed the most frequent genetic changes. The line in the middle of the profile indicates the baseline ratio (1.0), and the left and the right lines indicate ratio values of 0.85 and 1.17, respectively. **Left:** The profiles represent the following aberrations: gain of 7p21-pter (anaplastic carcinoma case 7), loss of entire chromosome 13 (medullary carcinoma case 9), and loss of entire chromosome 22 (follicular carcinoma case 15). **Right:** The profiles of chromosomes with no aberrations obtained from various negative control experiments.

tions were found in any of the 26 tumors examined, 13 (65%) of the 20 follicular carcinomas showed a deletion ($P < 0.0001$). Also, gains were more common in follicular than in papillary carcinoma (50% versus 12%, respectively; $P = 0.008$). In three follicular carcinomas, gains of multiple chromosomal regions were found (cases 6, 19, and 20, Table 1).

Deletion of chromosome 22 was particularly common in follicular carcinoma. The whole chromosome 22 was deleted in 7 (35%) carcinomas, and in addition, deletion of 22q12.3-qter was found in one carcinoma. This is a distinctive feature as compared with papillary carcinoma, where none of the cases had deletion of chromosome 22 ($P = 0.001$). Deletion of the entire chromosome 22 was present in 7 (54%) of the 13 widely invasive tumors, but in none of the 7 minimally invasive cases ($P = 0.04$). Deletion of chromosome 22 was also more common in older than in younger patients ($P = 0.01$). Four patients with follicular carcinoma were known to have died from thyroid cancer, and three of them had deletion of chromosome 22.

Figure 2. **Top:** Summary of gains and losses found in 26 papillary thyroid carcinomas. **Bottom:** Gains and losses of DNA sequences in 20 follicular thyroid carcinomas. The gains are shown on the right side of the chromosomes, and the losses are on the left. Each line represents genetic aberration seen in one tumor.

To confirm the deletions of chromosome 22 found in CGH analyses, five follicular carcinomas with a deletion in a CGH analysis (cases 9, 11, 13, 14, and 15, Table 1) and three follicular carcinomas where chromosome 22 was not found to be deleted (cases 1, 2, and 8) were analyzed by two-color FISH. In all five cases with a deletion of chromosome 22 in a CGH analysis, only one hybridization signal for both chromosome-22-specific probes was observed in more than 83% of the cells, whereas two signals were found in all three cancers that did not show the deletion in CGH. Hence, the FISH results were in line with the CGH findings.

In follicular carcinoma, a DNA copy number loss was common also in the long arm of chromosome 13 (five tumors, 25%) and in the short arm of chromosome 1 (four tumors, 20%; Figure 2, bottom). The minimal common region in the chromosome arm 13q was 13q21–22. In the chromosome arm 1p the minimal common region was

Figure 3. Top: Summary of gains and losses in 10 medullary thyroid carcinomas. Bottom: Gains and losses of DNA sequences in 13 anaplastic thyroid carcinomas. The gains are shown on the right side of the chromosomes, and the losses are on the left.

1p21–22. The most common regions with an increased DNA copy number were chromosomes 1q (5 tumors, 25%) and 17q (4 tumors, 20%). In one case, two high-level amplifications were detected, one at 14q11.2–q22 and another at 18q12.1–q21 (case 19, Table 1). Widely invasive follicular carcinomas appeared to have more DNA copy number changes than the minimally invasive ones (median, two *versus* one, respectively; $P = 0.06$).

Medullary Carcinoma

Five (50%) of the ten medullary carcinomas showed DNA copy number changes that occurred in eight different chromosomes (Figure 3, top). The median number of changes per tumor was 0.5 (range, 0 to 5). Four of the five tumors with copy number changes had deletions, and one had gains. A loss of an entire chromosome (chromosome 3, 13, or 22) was present in four cases, two of which showed a loss of chromosome 22 (20%). Losses of 1p31.1–p32, 18p, or 18q21–qter were each detected in one case. Multiple gains were found in one case (Table 2).

Anaplastic Carcinoma

Of the 13 anaplastic carcinomas, 11 (85%) showed DNA copy number changes (Figure 3, bottom). The total number of changes detected was 32, but in one case as many as 13 changes were detected (case 13, Table 2). Gains ($n = 27$) were more frequent than losses ($n = 5$). The most common aberration was a gain of 7p22–pter, which was found in four cases (31%). Other common gains were 8q22–qter ($n = 3$, 23%) and 9q34–qter ($n = 3$). Loss of chromosome 22 was found in one case (8%).

Comparison of CGH Findings between the Histological Types

A summary of the collected data are shown in Table 3. Among the 69 tumors investigated, a total of 100 DNA copy number changes were found. The majority ($n = 64$) were gains of genetic material. Gains were found in all histological types of thyroid cancer, but they were rare in the papillary and medullary types. Deletions were particularly common in follicular carcinoma (65%), and they were entirely absent in the papillary type. Both deletions and gains were found in 25% of follicular and 15% of anaplastic carcinomas, whereas none of the papillary or medullary carcinomas had both deletions and gains. Of particular interest is the deletion of chromosome 22, which was found frequently in follicular carcinoma (7 of 20, plus one partial deletion) and occasionally in medullary and anaplastic carcinomas.

Discussion

The striking differences in the DNA copy number changes between papillary and follicular thyroid carcino-

Table 3. Comparison of DNA Copy Number Changes in Thyroid Carcinoma by the Histological Type

Histological type of thyroid carcinoma	Number of changes found					Number of tumors with changes				
	Deletions	Gains	Any change	Median	Range	Deletions	Gains	Both deletion and gain	Deletion of chromosome 22	Any change
Papillary, $n = 26$	0/26	6/26	6/26	0	0–4	0 (0%)	3 (12%)	0 (0%)	0 (0%)	3 (12%)
Follicular, $n = 20$	22/20	26/20	48/20	2	0–8	13 (65%)	10 (50%)	5 (25%)	8 (40%)	16 (80%)
Medullary, $n = 10$	9/10	5/10	14/10	0.5	0–5	4 (40%)	1 (10%)	0 (0%)	2 (20%)	5 (50%)
Anaplastic, $n = 13$	5/13	27/13	32/13	2	0–13	3 (23%)	10 (77%)	2 (15%)	1 (8%)	11 (85%)

mas is intriguing. Both of these types of thyroid carcinoma are associated with generally favorable prognosis, and yet, as many as 80% of follicular carcinomas and only 12% of papillary carcinomas showed DNA copy number changes. Moreover, deletions were much more frequent in follicular than in papillary carcinoma (65% *versus* 0%, respectively). In particular, loss of chromosome 22 was common in follicular carcinoma, absent in papillary carcinoma, but occurred occasionally in medullary and anaplastic carcinomas. Both follicular and papillary carcinomas may contain follicles, and both tumors are expected to arise from follicular cells, but they differ considerably at the chromosomal DNA copy number level, suggesting that the genetic evolution of follicular and papillary carcinomas is different.

Clonal chromosomal abnormalities have been present in almost one-half of the cytogenetically examined papillary thyroid carcinomas by other methods than CGH. The most characteristic change has been an intrachromosomal rearrangement, a paracentric inversion in 10q, which has often been the only change found and which is not detectable by CGH.^{11-15,17,38-40} In the present series, CGH abnormalities in papillary carcinoma were associated with old age. Although such changes may be associated with the general decrease of genomic stability with age, this finding is well in line with earlier studies. Papillary carcinomas that occur in elderly patients have been found to be associated with more nuclear irregularity, a slightly increased mitotic rate, increased probability of DNA aneuploidy as determined by DNA flow cytometry, and greater mortality from thyroid cancer than papillary carcinomas of younger patients.^{41,42}

A deletion of the entire chromosome 22 or part of it was present in 40% of follicular carcinomas. Deletion of the entire chromosome was present only in the widely invasive type of follicular carcinoma, and three of the four patients who died of follicular carcinoma had this change. This suggests that loss of chromosome 22 may have prognostic value in the disease. Deletions have earlier been found in follicular carcinoma in a karyotype study.¹² A monosomy or a DNA copy number loss of chromosome 22 has also been described in some other human neoplasms, such as meningioma and mesothelioma.^{43,44} One candidate for the tumor suppressor gene in chromosome 22q is neurofibromatosis type 2 (NF2) at 22q12,⁴⁵ and there may be another putative suppressor gene distal to NF2.⁴⁶ The significance of these and other suppressor genes located in 22q in the genesis of follicular thyroid carcinoma is currently not known.

Chromosome 22 deletion found by CGH could be confirmed in all five cases studied by FISH, whereas none of the three cases where the deletion was not found in a CGH analysis showed chromosome 22 deletion by FISH. Hence, the results obtained by the two techniques were fully concordant. However, small DNA deletions or amplifications cannot be detected by CGH, and its ability to detect small DNA copy changes becomes worse as more of the tumor DNA used in the CGH analysis originates from normal host cells, which are always present when tumor tissue samples are used as the starting material. Although we made an effort to use representative tissue

samples, and we estimate that a maximum of 30% of the tumor DNA was derived from tumor-containing normal host cells, some of the smaller DNA copy number changes may have remained undetected because of the presence of normal cell DNA among the cancer cell DNA used in hybridizations.

Also, many other changes apart from the loss of chromosome 22 may be of importance in the genesis of follicular carcinoma. We found DNA loss in chromosome 13 in 25% of the cases. Deletions of 13q are present in various other neoplasms, such as retinoblastoma, prostate cancer, and renal cancer.⁴⁷ Many breast carcinomas have 13q deletion,⁴⁸ and DNA copy number losses in 13q, including the RB1 locus at 13q14, have been found in as many as 86% of small-cell lung carcinomas.⁴⁹ Similarly, we found loss of chromosome 1p in 20% of follicular carcinomas, and the loss of genetic material from 1p is a well established phenomenon in several cancers, although no significant tumor suppressor gene has as yet been identified in this region.⁵⁰ The most common gains in the present series were in 1q and 17q. Over-representation of chromosome 1q is common, eg, in mesothelioma, large-B-cell lymphoma, and bladder cancer.^{44,51,52} Chromosome 17q harbors the well known proto-oncogene ERBB-2.

Anaplastic thyroid carcinoma is one of the most aggressive human cancers, leading almost invariably to death within 2 to 3 years from the diagnosis, and one might expect to find numerous and chaotic genetic changes particularly in this tumor type. Although DNA copy number changes were demonstrated in most carcinomas (85%), the number of deletions was even lower than in follicular carcinomas (23% *versus* 65%, respectively). Comparison of the DNA profiles of follicular and anaplastic carcinomas suggests that the extent of DNA copy number changes does not directly correlate with the clinical aggressiveness of cancer, at least when different histological types of cancer are compared with each other (Figures 2 and 3). This hypothesis is supported by another study, where large gains of genetic material were found in many histologically and clinically benign thyroid adenomas by CGH.²⁷ Only few anaplastic carcinomas with an abnormal karyotype have been reported,^{12,28} and no characteristic cytogenetic patterns have been found. It has been suggested that some anaplastic carcinomas might have complex karyotypes that include signs of gene amplification with double minute chromosomes.²⁸ Our results may not support this, because we found high-level amplifications neither in anaplastic carcinoma nor in other types of thyroid cancer (the red-to-green ratio never exceeded 1.5). The most common changes in our series were gains in 7p22-pter, 8q22-qter, and 9q34-qter in anaplastic carcinoma. Some candidate proto-oncogenes found in these regions include the platelet-derived growth factor- α polypeptide gene (7p), the MYC gene (8q), and the ABL and VAV2 genes (9q).

Losses were more frequent than gains in medullary carcinoma, and one-half of the cases showed no changes in CGH analysis. This result, although based on a limited number of cases only, is in line with karyotype studies, where the most consistent finding has been a

normal modal number of chromosomes with a marked tendency to random hypodiploidy.⁵³ Hypodiploidy has been found also in cell lines established from medullary carcinoma.⁹ In the present study, genetic losses were most common in chromosomes 3, 13, and 22 (two losses in each). Apart from chromosome 3, these chromosomes showed frequent losses also in follicular carcinoma (Figures 2 and 3). No changes were detected in any of the cases in 10q at the location of the RET proto-oncogene.

We conclude that DNA copy number changes are frequent in follicular, medullary, and anaplastic thyroid carcinoma, but rare in papillary carcinoma when studied by CGH. Only gains of the genetic material were found in papillary carcinoma, and they were found only in elderly patients. Unlike in papillary carcinoma, extensive loss of genetic material occurs frequently in follicular carcinoma. Loss of chromosome 22 is particularly common in follicular carcinoma, but this change may be found also in medullary and anaplastic carcinomas. Loss of chromosome 22 is associated with the widely invasive histological type in follicular carcinoma, and its association with prognosis requires additional studies. The large differences in the CGH profiles between papillary and follicular carcinomas lend further support to the concept that papillary and follicular carcinomas are different entities and suggests that their genetic evolution is different.

Acknowledgment

We thank Ms. Elina Roimaa for skillful technical assistance.

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