Loss of heterozygosity in 73 human thyroid tumors

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Abstract

OBJECTIVES: The aim of the study was to establish the LOH frequency of selected polymorphic markers in different histological types of thyroid tumors: 18 colloid goiters (CG), five follicular adenomas (FA), nine follicular carcinomas (FTC), 40 papillary carcinomas (PTC), and one anaplastic carcinoma (ATC). For PTC, tumors negative for RET/PTC rearrangements were preferred.

METHODS: LOH studies were performed using 14 highly polymorphic markers previously described as frequently lost in thyroid tumors.

RESULTS: In 20 cases (27%) the loss of at least one marker was found. No difference between the frequency of the LOH in FTC and PTC tumors was revealed (33% v. 33%). No differences between histopathological subtypes of PTC in LOH were found. Papillary thyroid carcinomas showed a tendency to higher LOH frequency from patients older than 45 years of age compared to younger ones (9/23 v. 4/17) although it was not statistically significant.

CONCLUSIONS: We conclude that papillary thyroid cancers, particularly those diagnosed in patients older than 45 years of age, do exhibit LOH at least with the same frequency as follicular cancers. This increased number of LOH events may contribute to the clinical aggressiveness of cancer in older patients.

Introduction

Various studies have been performed to identify chromosomal regions harboring tumor suppressor gene (TSG) involved in thyroid tumorigenesis. Most authors used loss of heterozygosity (LOH) technique to detect allelic deletion. There was not much known about LOH in colloid goiters. Several chromosomal regions, such as 2p, 2q, 3p, 7q, 10p, 10q 11q, 13q, 17p and 17q, have been reported as frequently lost in thyroid adenomas and carcinomas [1–8]. Some data have proven that...
LOH on 7q21.1 and 7q31.1 were characteristic events in FA/FTC but did not occur in PTC [5]. Most authors indicated that LOH was more frequently observed in FTC than in PTC [4]. Also, correlation between histopathological subtypes as well as clinical data and LOH changes in thyroid neoplasm were noticed but the data were contradictory [2, 5, 8].

RET and TRK1 protooncogenes encoded for tyrosine kinase receptors are frequently involved in cytogenetic rearrangements leading to the formation of chimeric oncogenes [9]. The presence of RET rearrangements has ranged between a few and more than 80% of PTC cases, with the highest estimates in populations of patients with known radiation history [10]. Both types of rearrangements may alternatively constitute an initiating step in PTC development, while the nature of initiating event in RET/PTC and TRK1 negative PTC has still not been elucidated.

The aim of our study was to analyze the occurrence of LOH at different loci in human thyroid tumors and to estimate possible relationship between LOH and basic parameters such as histological type, stage and differentiation.

Material and methods

Tissue samples. The study group consisted of 73 thyroid tumors: 18 CG, 5 FA, 9 FTC, 40 PTC and one AC, diagnosed and classified according to WHO criteria [11]. The group of patients included 60 women and 13 men, ranging in age from 14 to 77 years (median 52±14), treated by thyroidectomy and, when indicated, neck modified lymphadenectomy.

For DNA extraction tumor fragments as well as normal tissue or blood were obtained from the same patients. Genomic DNA was prepared using standard procedure based on ionic detergent lysis and proteinase K digestion, phenol-chloroform extraction and isopropanol precipitation. The DNA concentration was quantitated by spectrophotometry (OD260/OD280 nm).

PCR and microsatellite analysis. LOH was detected by PCR-based 14 polymorphic microsatellite markers (Table 1). Primer sequences, cytogenetic localization and heterozygosity rate of markers were obtained from NCBI (http://www.ncbi.nlm.nih.gov). For the PCR amplification the forward primer was modified with 6-FAM, TET or HEX molecule (IDT Integrated DNA Technologies Inc. Coralville, IA, USA). Standard PCR reactions were carried out in a final volume of 15µl containing 50 ng of genomic DNA and the following concentrations: 0.33µM of each primer, 0.25mM dNTPs (Promega Corp. Madison, WI, USA), 2.5mM magnesium chloride and 0.04U/µl of Taq DNA Polymerase (MBI Fermentas). All PCR products were amplified using a PTC-200 Peltier Thermal Cycler (MJ Research, Watertown, MA, USA) under standard conditions. PCR products were prepared for capillary electrophoresis, with GenScan-500 TAMRA used as a size standard marker, according to the Applied Biosystem protocols (Applied Biosystem, Foster City, CA, USA). After denaturation, samples were analyzed in Genetic Analyzer ABI310 using ABI PRISM 310 Data Collection Software v 1.0.2. and ABI PRISM GeneScan Analysis Program. (Applied Biosystem, Foster City, CA, USA).

Determination of LOH. Definition of the allelic deletion was limited to informative (heterozygous) cases. Allelic loss was calculated using a normalized allele ratio (LOH ratio) equation: LOH=(T1)(N2)/(T2)(N1) where N1 and N2 are peaks from normal DNA and T1 and T2 are peaks from tumor DNA. Marker in which the LOH ratio was below 0.6 was scored as lost.

Determination of RET/PTC rearrangements. Data for RET/PTC rearrangements were available for 31 PTC tumors. For RET rearrangements study, mRNA specimens were extracted from frozen tissues using Dynabeads mRNA DIRECT™ Kit (Dynal, Oslo, Norway) and then reverse-transcribed (cDNA Cycle Kit; Invitrogen, CA, USA). 2 µl of cDNA were amplified during PCR I to check whether any RET rearrangement is present according to the method described by Klabueier et al. [12]. The PCR II was performed to amplify specific transcripts for 7 RET/PTC types (PTC1-7). PCR products were electrophoresed in 5% PAGE gels and stained with ethidium bromide.

Statistical analysis. For statistical analysis the Fisher exact test was used.

Results

The complete list of markers used in the study and the LOH results for each polymorphic marker are shown in Table 1.

The percentage of informative loci varied from 50–100% of all cases analyzed. In 20 cases (27%) the loss of at least one marker was found. Marker D10S607 (10q22.2) was the most frequently lost in the whole group of thyroid tumors (6/49–13%). The second most often deleted marker was D7S486 (7q31.1–32) gene locus) (5/58–9%). Five of 40 PTC cases (13%) and the anaplastic carcinoma sample presented LOH in multiple sites in different chromosomes. One case of 18 (6%) CG showed LOH in marker localized on 10q22.2 (D10S607). In two of five FA (40%) losses were found in two markers: D7S660 (7q21.1–1/5) and D10S607 (1/3). In follicular carcinoma LOH was present in three cases (3/9–33%), in markers D7S660 (1/8), D10S607 (1/7) and D17S520 (17p12–1/7). In the one analyzed case of anaplastic cancer, loss of two markers was found (D3S1435 and D7S660).

13 of 40 papillary carcinomas (33%) revealed LOH in at least one locus. In PTC we observed that losses were more frequently present in group of patients older than 45 years of age compared with younger ones (9/23 v. 4/17) but no statistical significance was found. Detailed results obtained in the above two groups are presented in Table 2. The differences in deleted loci in both groups were noticed. In the older group of patients the marker most frequently lost was D7S486 (22%–4/18). In the group of younger patients marker D17S520 (17p12) was lost in two out of 13 tumors.

For 31 PTC tumors the corresponding mRNA was available and the analysis of RET/PTC rearrangements...
Loss of heterozygosity in 73 human thyroid tumors was performed. Their absence was revealed by a RT-PCR study. Among confirmed RET/PTC negative PTC cases the frequency of LOH-positive tumors was 39% (12/31), with 4/16 (25%) cases diagnosed in patients under 45 years of age and 8/15 (53%) in older PTC patients.

Discussion

Loss of genetic material in thyroid neoplasm has been studied using cytogenetic methods as well as LOH techniques. Different chromosomal regions have been suggested to be involved in the development of thyroid cancer, but the findings are still controversial. We used LOH technique to analyze markers which were suggested by other authors to be specifically lost in different histological types of thyroid tumors.

In our studies only one case of colloidal goiter showed LOH in marker localized in 10q22.2 (D10S607). To our knowledge this is the first case of CG which reveals any LOH. Interestingly, marker D10S607 was most frequently lost in different subtypes of thyroid tumors analyzed (6/48–13%), which may suggest the early role of the TSG localized at this loci.

Several authors found that LOH at 10q22–24 was more frequently observed in FA than in FTC, suggesting that there might be two different pathways in developing thyroid follicular tumors [1,6]. Our results displayed the same phenomenon although low number of informative cases prevented us from performing statistical analysis.

Table 1: Localization of the chromosome markers used in this study of 73 human thyroid tumors and summary of LOH versus informative cases in each locus studied.

<table>
<thead>
<tr>
<th>Locus symbol</th>
<th>Band location</th>
<th>CG (n=18)</th>
<th>FA (n=5)</th>
<th>FTC (n=9)</th>
<th>PTC (n=40)</th>
<th>AC (n=1)</th>
<th>total (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYCL</td>
<td>1p32</td>
<td>0/14</td>
<td>0/5</td>
<td>0/7</td>
<td>1/26 (4)</td>
<td>0/1</td>
<td>1/53 (1.9)</td>
</tr>
<tr>
<td>D2S123</td>
<td>2p16</td>
<td>0/15</td>
<td>0/3</td>
<td>0/7</td>
<td>2/25 (8)</td>
<td>0/1</td>
<td>2/51 (3.9)</td>
</tr>
<tr>
<td>D2S1328</td>
<td>2q14.3</td>
<td>0/11</td>
<td>0/2</td>
<td>0/6</td>
<td>3/26 (12)</td>
<td>nd</td>
<td>3/45 (6.7)</td>
</tr>
<tr>
<td>D3S1101</td>
<td>3p12</td>
<td>0/16</td>
<td>0/5</td>
<td>0/9</td>
<td>1/32 (3)</td>
<td>0/1</td>
<td>1/63 (1.6)</td>
</tr>
<tr>
<td>D3S1481</td>
<td>3p14.2</td>
<td>0/13</td>
<td>0/5</td>
<td>0/9</td>
<td>1/32 (3)</td>
<td>0/1</td>
<td>1/60 (1.7)</td>
</tr>
<tr>
<td>D3S1435</td>
<td>3p24.2</td>
<td>0/14</td>
<td>0/4</td>
<td>0/7</td>
<td>0/26</td>
<td>1/1 (100)</td>
<td>1/52 (1.9)</td>
</tr>
<tr>
<td>D7S665</td>
<td>7p15</td>
<td>0/13</td>
<td>0/3</td>
<td>0/5</td>
<td>1/29 (3)</td>
<td>0/1</td>
<td>1/51 (2)</td>
</tr>
<tr>
<td>D7S660</td>
<td>7q21.1</td>
<td>0/10</td>
<td>1/5 (20)</td>
<td>1/7 (14)</td>
<td>0/22</td>
<td>1/1 (100)</td>
<td>3/45 (6.7)</td>
</tr>
<tr>
<td>D7S492</td>
<td>7q21.1</td>
<td>0/16</td>
<td>0/3</td>
<td>0/7</td>
<td>1/24 (4)</td>
<td>0/1</td>
<td>1/51 (2)</td>
</tr>
<tr>
<td>D7S486</td>
<td>7q31.1</td>
<td>0/16</td>
<td>0/5</td>
<td>0/8</td>
<td>5/28 (18)</td>
<td>0/1</td>
<td>5/58 (8.6)</td>
</tr>
<tr>
<td>D10S607</td>
<td>10q22.2</td>
<td>1/13 (8)</td>
<td>1/3 (33)</td>
<td>1/6 (17)</td>
<td>3/26 (12)</td>
<td>nd</td>
<td>6/48 (12.5)</td>
</tr>
<tr>
<td>D15S907</td>
<td>15q11.2</td>
<td>0/13</td>
<td>0/2</td>
<td>0/4</td>
<td>1/28 (4)</td>
<td>0/1</td>
<td>1/48 (2.1)</td>
</tr>
<tr>
<td>D17S520</td>
<td>17p12</td>
<td>0/16</td>
<td>0/4</td>
<td>1/7 (14)</td>
<td>2/33 (6)</td>
<td>nd</td>
<td>3/60 (5)</td>
</tr>
<tr>
<td>TP53</td>
<td>17p13.1</td>
<td>0/10</td>
<td>0/4</td>
<td>0/5</td>
<td>0/22</td>
<td>0/1</td>
<td>0/47</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1/18 (5.6)</td>
<td>2/5 (40)</td>
<td>3/9 (33)</td>
<td>13/40 (33)</td>
<td>1/1 (100)</td>
<td>20/73 (27)</td>
</tr>
</tbody>
</table>


Table 2: The comparison of LOH frequency between patients of papillary carcinoma diagnosed under and over 45 years of age

<table>
<thead>
<tr>
<th>Number of cases with LOH in</th>
<th>PTC (n=40)</th>
<th>RET/PTC negative (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>one locus</td>
<td>two loci</td>
<td>three or more loci</td>
</tr>
<tr>
<td>P=0.298</td>
<td>P=0.1061</td>
<td></td>
</tr>
<tr>
<td>under 45 yrs (n=17)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>over 45 yrs (n=23)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>under 45 yrs (n=16)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>over 45 yrs (n=15)</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>
In studies performed by Trovato et al. [5] four markers (D7S660, D7S492 from 7q21.1 and D7S486, D7S655 from 7q31.1) were lost in all FTC studied and in 10–20% of FA cases, whereas no losses in PTC were observed. It was suggested that the above-mentioned markers could be used for distinguishing FA/FTC from PTC. These authors also found the 100% prevalence of LOH of 7q markers in ATC cases, which could prove that ATC and FTC shared genetic abnormalities and that there were two subsequent stages of the same pathological pathway [5]. Our results as well as other data did not confirm the observation that 7q markers were selectively lost in FTC but not in PTC. [7,8,13]. In both FA and FTC we found LOH (2/12–17%) only in marker D7S660 (7q21.1), while in PTC allelic losses were found in two markers – D7S486 (5/28–18%) and D7S492 (1/24–4%). These results suggest that these markers are not characteristically lost in FA/FTC subtypes. Since consistent losses of markers localized in chromosome 7q were described by many authors it was suggested that at least one TSG is localized in 7q in the vicinity of the METC locus [5,7,8].

It has been reported that allelic losses in FA and FTC (33% and 60% cases) were significantly more frequent than in PTC (23%) [4]. Other studies seemed to prove the above results [1,2,3,8]. In our studies the difference between the frequency of the LOH in FTC and PTC was not found (33% vs. 33%). In order to analyze the possible reasons of the higher frequency of the LOH observed by us, we inspected the influence of age at PTC diagnosis and of the RET mutations occurrence.

RET rearrangements are found in 6–84% cases of PTC, depending on the population investigated, radiation exposure and method of estimation. In average, 25–30% PTC are RET-positive, with the higher incidence in younger PTC patients. In our experience the frequency of RET mutations (mainly intrachromosomal inversions) in the Polish population of PTC approaches 50% in patients younger than 30 years of age at diagnosis while it is several times lower (under 10%) in patients diagnosed with PTC after the age of 50 [14]. The age-dependence of RET mutations has been reported by others [15,16]. On the other hand, others relate the occurrence of RET/PTC mutations only to radiation-induced PTC [12]. Molecular events leading to the development of PTC in the absence of RET or TRK rearrangements are largely unknown. Recent reports indicate on the high frequency of BRAF mutations which may lead to the conclusion that the activation of the alternate steps of the tyrosine kinase receptors-RAS-MAPK pathway may result in development of papillary thyroid cancer [17,18]. However, the impact of other, still unrecognized genes is also discussed and our observation, that RET/PTC negative tumors exhibit a nearly 40% frequency of LOH, may help to localize the potential TSG involved. This mechanism may preferentially operate in older PTC patients, in whom the clinical symptoms of malignancy are more distinctly expressed and more often lead to a fatal outcome [19,20]. As RET rearrangements were excluded in majority of PTC cases in this study, other molecular events leading to the development of PTC could be easier detected. We did not find any information about the occurrence of RET rearrangements in other studies of LOH in PTC.

Using CGH analysis, some authors displayed an increasing number of alterations accompanied by increased aggressiveness of thyroid cancer, but this relationship was present only in papillary not follicular tumors [21,22]. In another report, LOH events were observed more frequently in cases deceased of papillary carcinoma [23]. We did not correlate our results with the clinical course of the disease, as the number of patients with very advanced PTC was rather small. Instead, we performed a comparison of the LOH occurrence in two age groups of PTC (under and above 45 years of age). In the group of older PTC patients allelic deletions were present more frequently (39% vs. 23%) but it was not statistically significant. In relative terms, the last difference was even more distinct, when proven RET/PTC-negative tumors were considered – a twofold increase was seen in older patients. It is known, that the prognosis is much poorer in PTC patients older than 40–50 years of age [19,20]. Thus, the increased number of LOH events may contribute to the clinical aggressiveness of cancer in older patients, especially in those papillary thyroid tumors which are not initiated by a RET/PTC rearrangement. In conclusion, our study shows that papillary thyroid cancers, particularly those diagnosed in patients older than 45 years of age, do exhibit LOH at least with the same frequency as follicular cancers.

Acknowledgments

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Loss of heterozygosity in 73 human thyroid tumors

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