Cytogenetic Changes in Radiation-induced Tumors of the Thyroid


ABSTRACT

Thyroid carcinoma incidence is increased significantly after ionizing irradiation; however, the possible mechanisms have not yet been identified. To provide clues for an understanding of the radiation-induced transformation of thyroid epithelium, we analyzed the karyotypes of 56 childhood thyroid tumors that appeared in Belarus after the Chernobyl nuclear accident in 1986. We also studied eight secondary thyroid tumors that developed after radiotherapy. Metaphase preparations obtained from primary cultures were analyzed by G-banding. Clonal structural aberrations were found in 13 of 56 Belarussian cases and in 6 of 8 secondary tumors that developed after radiotherapy. Furthermore, we detected multiple chromosomal aberrations as well as complex rearrangements in some of these tumors and performed a detailed analysis of marker chromosomes from a single case using spectral karyotyping and comparative genomic hybridization in a childhood tumor from Belarus with a near-triploid karyotype. Both comparative genomic hybridization and spectral karyotyping analysis revealed structural alterations affecting identical chromosomes 1, 2, 9, and 13, among others. In addition to the known hot spots of alterations in papillary thyroid carcinomas on chromosomes 1q and 10q, a comprehensive breakpoint analysis in the pooled data set revealed novel breakpoints on chromosomes 4q, 5q, 6p, 12q, 13q, and 14q. The chromosomal aberrations in these tumors may provide suitable starting points for the positional cloning of genes involved in radiation-induced tumorigenesis.

INTRODUCTION

The release of radiiodine in the aftermath of the Chernobyl nuclear power plant accident in April 1986 led to an unprecedented exposure of the thyroid glands of inhabitants in the affected regions of Belarus, Ukraine, and Russia. Iodine is incorporated through the diet and is rapidly absorbed in the intestine as inorganic iodide. Serum iodide is rapidly absorbed in the intestine as inorganic iodide. Serum iodide is concentrated by the thyroid gland through an active transport mechanism and is incorporated in the protein thyroglobulin to form iodotyrosines, the precursors of thyroid hormones (1). As early as 4 years after the accident, the incidence of childhood PTCs in the most contaminated region, the region of highest contamination. Fourteen papillary carcinoma cases occurred in the Brest region, seven cases occurred in the Minsk region, and seven cases occurred in four other regions. As a reference group, eight secondary thyroid tumors that developed after earlier radioiodine therapy or external radiotherapy (age at surgery, 28–82 years; five adenomas, one medullary carcinoma, and two papillary carcinomas) were received from the Martha-Maria Hospital (Munich, Germany). Corresponding nontumorous thyroid tissues were received with tumor tissue when available.

Tissue Culture and Chromosome Analysis. Tissue culture and chromosome analysis were performed as described previously (20, 22). In brief, disaggregated tissues were seeded directly onto glass slides, and chromosome preparations were carried out without further passage if possible. The epithelial nature of cultured cells was assessed by immunocytochemical staining of anticytokeratin (AE1/AE3; Boehringer Mannheim). Chromosome preparations were carried out after an in vitro culture of cells for 8–21 days. After G-banding with Wright’s staining solution, karyotyping was performed according to the International System for Human Cytogenetic Nomenclature (23).

Three cell lines from S48, a highly complex rearranged childhood case from Belarus, were successfully cloned and also karyotyped by G-banding.

SKY. For SKY analysis of S48, chromosome preparations were aged for 2 weeks. Metaphase preparations were pretreated with RNase A (0.1 mg/ml in

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The abbreviations used are: PTC, papillary thyroid carcinoma; SKY, spectral karyotyping; CGH, comparative genomic hybridization; DAPI, 4',6-diamidino-2-phenylindole;
2× SSC) and pepsin solution (12 μg/ml of 0.01 M HCl) and fixed in 1% formaldehyde. Pepsin digestion was performed under microscopic control, and slides were placed in denaturing formamide (70% formamide in 2× SSC) at 72°C for 90 s and dehydrated in a 70, 80, and 100% ethanol series. They were subsequently hybridized with a probe mixture supplied by Applied Spectral Imaging, Inc. (San Diego, CA). The probe mixture contains 24 painting probes that are specific for each human chromosome and labeled with combinations of five different fluorescent dyes (Spectrum Green, Spectrum Orange, Texas Green, Texas Orange, and Texas Red). This hybridization solution was applied to slides of five different fluorescent dyes (Spectrum Green, Spectrum Orange, Texas Green, Texas Orange, and Texas Red) and covered with antifade solution (Vectashield Mounting medium; Vector Laboratories, Burlingame, CA). Metaphases were acquired using a SpectraCube system (Applied Spectral Imaging, Inc.) and analyzed with SKYView imaging software (24).

**RESULTS**

A total of 949 karyograms from 56 childhood thyroid tumors from Belarus as well as 167 karyograms from 8 secondary thyroid tumors that developed after radiotherapy were investigated for chromosomal aberrations. A total of 308 abnormal karyotypes (32%) were detected in both tumor groups. A total of 13 Belarusian cases (23%) and 6 secondary tumors (75%) showed clonal structural chromosome aberrations (Tables 1 and 2); in an additional Belarussian adenoma (S76), two clones with numerical aberrations (45,XX,−19 and 45,XX,−22) were found. In Belarusian cases as well as in secondary tumors, multiple chromosomal changes within one cell were frequently detected (e.g., cases S9, S48, S65, S61, S76, S79, S112, S115, S125, and S126; Tables 1–4). Two cases (S9 and S48) exhibited very complex structural rearranged chromosomes that could not adequately be described by G banding. One of the cell lines derived from S48 was also studied by SKY and CGH. Using SKY, various derivative chromosomes were repeatedly detected in the five metaphases investigated (Fig. 1). The following karyotype resulted from these five metaphases: 46,XX,t(1;2)(q22;q34)/2,46,XX,t(4;11)(q31;q23), 46,XX,t(X;10)(q13;p12)/2,46,XX,del(1)(p32)/2,46,XX,del(1q11)/2,46,XX,t(1;2)(q42;p25)/2,46,XX,t(2;13)(q12)/2,46,XXX,t(2;12)(q23;q13)/2. CGH revealed chromosomal gains on chromosomes 1p34, 1q21, 2p11.1–pter, 2q11.2–q13 (high-level amplification), 3q26.2–qter, 3p26.2–p22, 4q25–qter, 5q31–qter, 6q25–qter, 7q11–q36, 8q24–qter, 9q31–qter, 10q24–qter, 11q22–qter, 12p13–p11, 13q14–qter, 14q32–qter, and 17q11–qter.

**Table 1** Clonal chromosome aberrations in childhood thyroid tumors from Belarus

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at exposure (yr)/sex</th>
<th>Age at surgery (yr)</th>
<th>Pathological diagnosis</th>
<th>Tumor classification</th>
<th>No. of metaphases studied</th>
<th>Aberrant karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>S48</td>
<td>37/m</td>
<td>52</td>
<td>Adenoma</td>
<td>Moderate</td>
<td>10</td>
<td>46,XX,del(1q11)/2</td>
</tr>
<tr>
<td>S65</td>
<td>59/m</td>
<td>6</td>
<td>Rhabdoid carcinoma</td>
<td>Moderate</td>
<td>17</td>
<td>46,XX,t(1;2)(p22;q34)/2,46,XX,del(1q11)/2,46,XX,t(1;2)(p22;q34)/2,46,XX,t(1;2)(q12;p13)/2,46,XX,t(1;2)(q12;p13)/2,46,XX,t(1;2)(q12;p13)/2</td>
</tr>
<tr>
<td>S9</td>
<td>53/m</td>
<td>6</td>
<td>Adenoma</td>
<td>Moderate</td>
<td>10</td>
<td>46,XX,del(1q11)/2</td>
</tr>
<tr>
<td>S106</td>
<td>14/f</td>
<td>6</td>
<td>Adenoma</td>
<td>Moderate</td>
<td>6</td>
<td>46,XX,t(1;2)(p22;q34)/2,46,XX,del(1q11)/2,46,XX,t(1;2)(p22;q34)/2,46,XX,t(1;2)(q12;p13)/2,46,XX,t(1;2)(q12;p13)/2,46,XX,t(1;2)(q12;p13)/2</td>
</tr>
</tbody>
</table>

*RI, radioiodine therapy; EX, external radiation therapy.

**Table 2** Clonal chromosome aberrations in secondary thyroid tumors after therapeutic irradiation

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at exposure (yr)/sex</th>
<th>Type of irradiation</th>
<th>Pathological diagnosis</th>
<th>Tumor classification</th>
<th>No. of metaphases studied</th>
<th>Aberrant karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>S9</td>
<td>53/m</td>
<td>RI</td>
<td>Adenoma</td>
<td>Moderate</td>
<td>24</td>
<td>46,XX,del(1q11)/2</td>
</tr>
<tr>
<td>S42</td>
<td>37/m</td>
<td>EX</td>
<td>PTC</td>
<td>Moderate</td>
<td>16</td>
<td>46,XX,del(1q11)/2</td>
</tr>
<tr>
<td>S55</td>
<td>82/f</td>
<td>RI</td>
<td>Medullary carcinoma</td>
<td>Moderate</td>
<td>17</td>
<td>46,XX,del(1q11)/2</td>
</tr>
<tr>
<td>S65</td>
<td>59/m</td>
<td>EX</td>
<td>PTC</td>
<td>Moderate</td>
<td>11</td>
<td>46,XX,del(1q11)/2</td>
</tr>
</tbody>
</table>

*RI, radioiodine therapy; EX, external radiation therapy.

Tumor-node-metastasis (TNM) classification according to Ref. 21. Translocations confirmed by fluorescence in situ hybridization painting for chromosomes 1, 6, and 10 in a higher number of metaphases.

2× SSC) and pepsin solution (12 μg/ml of 0.01 M HCl) and fixed in 1% formaldehyde. Pepsin digestion was performed under microscopic control, and slides were placed in denaturing formamide (70% formamide in 2× SSC) at 72°C for 90 s and dehydrated in a 70, 80, and 100% ethanol series. They were subsequently hybridized with a probe mixture supplied by Applied Spectral Imaging, Inc. (San Diego, CA). The probe mixture contains 24 painting probes that are specific for each human chromosome and labeled with combinations of five different fluorescent dyes (Spectrum Green, Spectrum Orange, Texas Green, Texas Orange, and Texas Red). This hybridization solution was applied to slides of five different fluorescent dyes (Spectrum Green, Spectrum Orange, Texas Green, Texas Orange, and Texas Red) and covered with antifade solution (Vectashield Mounting medium; Vector Laboratories, Burlingame, CA). Metaphases were acquired using a SpectraCube system (Applied Spectral Imaging, Inc.) and analyzed with SKYView imaging software (24).

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tumors, a type of tumor that is normally rare in childhood. This is the first extensive cytogenetic study of childhood thyroid tumors without radiation history. Besides the known hot spots of aberrations in PTCs on chromosomes 1q and 10q, novel breakpoints on chromosomes 4q, 5q, 6p, 12q, 13q, and 14q were found in Belarussian cases originating predominantly from Gomel, the region with the highest contamination. In 7 of 11 childhood cases from Belarus, the clonal structural abnormalities were found in either all (S47, S48, S96, S175, and S179) or a large number of the metaphases analyzed (S253 and S284). This might be indicative of largely homogeneous tumor cell populations, whereas other cases with smaller clones (S81, S95, S99, and S125) appear to be more heterogeneous with respect to their karyotypic patterns. Breakpoints detected in structural aberrations from Belarusian tumors coincide in part with those already described for tumors from adults without a radiation history. The combined application of SKY and CGH revealed common chromosomes (1q, 2p, 9q, and 13q) affected by translocations as well as by chromosomal gains and losses.

DISCUSSION

Karyotype abnormalities in childhood thyroid tumors from Belarus and from secondary thyroid tumors developed after radiotherapy were investigated by G-banding. One case exhibiting multiple chromosomal changes was also studied by SKY and CGH. To our knowledge, the first extensive cytogenetic study of childhood thyroid tumors, a type of tumor that is normally rare in childhood (i.e., age < 15 years at the time of surgery). In addition to the clonal structural aberrations detected in 13 of 56 (23%) childhood tumors from Belarus and in 6 of 8 (75%) secondary thyroid tumors developed after radiotherapy, additional nonclonal structural abnormalities were found in 10 Belarusian cases (19%) and 7 secondary tumors (88%; Tables 3 and 4).

Tumor-node-metastasis (TNM) classification according to Ref. 21.

Table 3 Non-clonal structural chromosome aberrations in childhood thyroid tumors from Belarus

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at surgery (yr/sex)</th>
<th>Age at exposure (yr)</th>
<th>Pathological diagnosis</th>
<th>Tumor classification</th>
<th>Differentiation</th>
<th>Total</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>S47 10/m 3 yr</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>Low</td>
<td>15</td>
<td>1</td>
<td>46,XY,7q+;9q-</td>
<td></td>
</tr>
<tr>
<td>S57 15/m 8 yr</td>
<td>PTC</td>
<td>pT_N2M0</td>
<td>Moderate</td>
<td>18</td>
<td>2</td>
<td>46,XY (t(7;7)(p21;q11))</td>
<td></td>
</tr>
<tr>
<td>S75 13/f 8 yr</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>High</td>
<td>15</td>
<td>1</td>
<td>46,XX,del(2)(p11)</td>
<td></td>
</tr>
<tr>
<td>S81 9/m 1 yr</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>High</td>
<td>14</td>
<td>4</td>
<td>46,XY (t(7;9)(q22;q33)/46,XY,del(2)(p13),del(9)(q13)/46,XY,inv(16))</td>
<td></td>
</tr>
<tr>
<td>S95 14/m 6 yr</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>High</td>
<td>12</td>
<td>6</td>
<td>46,XY,del(1p22)/46,XY (t(6;10)(q25:p13)/45,XY,del(19)(p13)(2);6;9)(p23</td>
<td>21)</td>
</tr>
<tr>
<td>S99 10/f 3 yr</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>High</td>
<td>16</td>
<td>9</td>
<td>46,XX,del(7)(q22)/46,XX,t(1;4)(q32;q31),t(4;8)(q21;p?)/46,XX,del(1q6)/46,XX,t(19)(p22)</td>
<td></td>
</tr>
<tr>
<td>S124 8/m 8 mo</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>Low</td>
<td>16</td>
<td>4</td>
<td>46,XY,del(10)/46,XY,del(9).</td>
<td></td>
</tr>
<tr>
<td>S125 9/m 11 mo</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>Low</td>
<td>23</td>
<td>11</td>
<td>46,XY,del(10)/46,XY,del(9)</td>
<td></td>
</tr>
<tr>
<td>S135 9/f 11 mo</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>High</td>
<td>10</td>
<td>2</td>
<td>46,XX,del(10)</td>
<td></td>
</tr>
<tr>
<td>S181 10/f 1 yr</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>High</td>
<td>16</td>
<td>2</td>
<td>46,XX,del(10)</td>
<td></td>
</tr>
</tbody>
</table>

q26.3, 5q23–q31, 6p21.3–pter, 7q11, 9q13–q33 (high-level amplification), 12q22–qter, 13q32–qter, 17q11.1–qter, 19, 20, and X and losses on 1q42, 13q21, and 15q11.1–q14. A combined application of SKY and CGH revealed common chromosomes (1q, 2p, 9q, and 13q) affected by translocations as well as by chromosomal gains and losses.

Pooled results of an overall breakpoint analysis of structural chromosome abnormalities in Belarusian tumors and in secondary tumors are summarized in Fig. 2 and compared with literature data on thyroid tumors without radiation history. Besides the known hot spots of alterations in PTCs on chromosomes 1q and 10q, novel breakpoints on chromosomes 4q, 5q, 6p, 12q, 13q, and 14q were found in Belarusian tumors and secondary tumors after radiotherapy.

Table 4 Nonclonal structural chromosome aberrations in secondary thyroid tumors after therapeutic irradiation

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at surgery (yr/sex)</th>
<th>Age at exposure (yr)</th>
<th>Type of irradiation</th>
<th>Pathological diagnosis</th>
<th>Tumor classification</th>
<th>Differentiation</th>
<th>Total</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>S97 10/m 10 yr</td>
<td>PTC</td>
<td>pT_N1M0</td>
<td>Moderate</td>
<td>18</td>
<td>6</td>
<td>46,XX,del(2)(p11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S76 20/f 4 EX</td>
<td>Adenoma</td>
<td>18</td>
<td>6</td>
<td>46,XX,del(2)(p11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S106 56/f 13 EX</td>
<td>Adenoma</td>
<td>11</td>
<td>8</td>
<td>46,XX,del(2)(p11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S121 66/f 63 RI</td>
<td>Adenoma</td>
<td>16</td>
<td>7</td>
<td>46,XX,del(2)(p11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* RI, radioiodine therapy; EX, external radiation therapy.
* Tumor-node-metastasis (TNM) classification according to Ref. 21.

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childhood carcinomas from Belarus. It is interesting to note that chromosomal band 10q11.2 harbors the RET proto-oncogene. In fact, a rearranged RET proto-oncogene has been reported in the majority of cases (27–29) as a very common event in childhood PTCs. In addition to these known structural chromosomal changes, breakpoints were detected in papillary childhood carcinomas from Belarus and in secondary tumors developed after radiotherapy (e.g., on chromosomes 4q, 5q, 6p, 12q, 13q, and 14q; Fig. 2), which have not been reported thus far to be involved in structural rearrangements of papillary cancers. Moreover, a large number of coincidental breakpoints were observed between childhood thyroid tumors from Belarus and secondary tumors developed after radiotherapy. However, the secondary thyroid tumors exhibited aberrations on Xq, 18, and 19p that were not detected in childhood cases from Belarus. Clusters of chromosomal breakpoints in radiation-induced tumors were visible on chromosomes 1, 4q, 6p, 10q, 12q, and 13q, irrespective of the tumor entity. The Belarusian childhood PTCs exhibited a breakpoint pattern that differs greatly from published data on adult papillary carcinoma without radiation history. In particular, chromosomal breakpoints on 4q, 5q, 6p, 12q, 13q, and 14q were observed in papillary childhood carcinomas but were never or only rarely observed in “spontaneous” papillary carcinomas. To our knowledge, no other cytogenetic reports of childhood thyroid carcinomas without radiation history and adult thyroid carcinomas from Belarus exist thus far for further comparison with the data from this study.

Although papillary carcinomas represent the vast majority of thyroid tumors after an exposure to ionizing radiation, there are some benign tumors among our subset of radiation-induced tumors (S9, S71, S76, S106, and S121) showing clonal numerical (S76) and structural chromosome aberrations. Four of these cases were diagnosed as adenomas (S9, S76, S106, and S121), whereas one childhood case was diagnosed as a goiter (S76). Structural chromosome abnormalities have been described previously in benign tumors, such as adenomas and goiter (30–34). Preferential 19q aberrations have been reported in follicular adenomas (31, 33) and also in a case of a multinodular goiter (32). In our cases of benign tumors, structural aberrations affecting 19q were completely absent, but one case (S9) showed a multiple aberration pattern, and other cases exhibited aberrations on chromosomes 1, 10, 15, and X.

SKY allows the painting of all chromosomes in different colors and is thus an excellent tool to analyze marker chromosomes and detect hidden chromosomal abnormalities, as demonstrated in hematological malignancies (35, 36). In this study, the application of SKY enabled a detailed analysis of marker chromosomes in the complex rearranged case S48, in which the chromosome number in the primary culture...
varied from near triploid (66–70 chromosomes) to tetraploid (92 chromosomes). It is interesting that both SKY and CGH demonstrated chromosomal changes on 1q, 2p, 9q, and 13q. This outlines the very complex rearrangements occurring in this particular case and points to potential hot spots for radiation-induced chromosomal damage.

In summary, this study presents cytogenetic findings in radiation-induced thyroid tumors (childhood thyroid tumors from Belarus and secondary tumors developed after radiotherapy). Multiple structural chromosome aberrations as well as complex rearrangements were more frequently detected in these tumors than in spontaneous thyroid tumors. In particular, this holds true for PTC, which is the predominant tumor entity among the childhood cases from Belarus. The radiation-induced tumors revealed some novel chromosomal breakpoints of structural aberrations (4q, 5q, 6p, 12q, 13q, and 14q) in addition to the changes already reported in spontaneous tumors (e.g., 1q and 10q). The report of novel breakpoints in radiation-induced thyroid tumors may serve as
a starting point for the characterization and positional cloning of genes involved in radiation-induced tumorigenesis.

REFERENCES


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