Cytogenetic Changes in Radiation-induced Tumors of the Thyroid


University of Munich, D-85758 Oberschleißheim, Germany [M. W.]; and Institute of Radiation Biology, Ludwig Maximilians University, D-80336 Munich, Germany [H. Z., E. L., A. M. K.]

INTRODUCTION

The release of radioiodine 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 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, Gomel, had increased drastically, exceeding 100 cases per million children per year (2–4).

PTC is the most common primary cancer of the thyroid gland (5, 6); however, it is very rare in children without a radiation history. It usually pursues an indolent course, but some cases in adults and particularly in children from Belarus exhibit a more aggressive behavior. Thus far, cytogenetic studies on PTC had been performed on adult cases (7–18), but not on childhood PTC. Published data from adult PTCs identified clonal structural chromosomal abnormalities often affecting chromosome 10q11.2 in almost one-half of the examined cases (8–11, 13, 14, 16–18). A relationship between this chromosomal breakpoint and thyroid tumorigenesis was further supported by the frequent finding of a truncated RET proto-oncogene in the same chromosomal band (19).

Previously, we have measured stable chromosomal translocations in the radiation-induced PTCs of 40 Belarusian children by chromosome painting of chromosomes 1, 4, and 12 and found an elevated translocation frequency in a subset of cases (20). However, chromosome painting for only a few selected chromosomes does not allow a detailed analysis of translocation breakpoints, and the relationship between the observed cytogenetic changes and radiation-induced thyroid tumorigenesis could not be investigated. In the study presented here, karyotype abnormalities were investigated by G-banding on 56 childhood thyroid tumors from Belarus and on 8 secondary thyroid tumors that developed after radiotherapy; the aim was to identify chromosomal changes associated with radiation-induced tumorigenesis in the thyroid gland.

MATERIALS AND METHODS

Tumor Specimens. A total of 56 thyroid tumors from Belarusian patients were collected between 1993 and 1996 on admission for surgery by the Center for Thyroid Tumors in Minsk, Belarus (36 females and 17 males; age at exposure, 0–7 years; age at surgery, 5–15 years; for 3 females, age at exposure ranged from 13–16 years and age at surgery ranged from 23–24 years). A total of 51 cases were classified as papillary carcinomas (21), 3 cases were classified as benign tumors, and 2 cases were classified as thyreoiditis. A total of 26 of 51 papillary carcinomas showed a high grade of differentiation, 13 of 51 papillary carcinomas showed a moderate grade of differentiation, and 12 of 51 papillary carcinomas showed a low grade of differentiation. Of the 56 Belarusian tumor cases, 28 occurred in the Gomel region, i.e., 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 analyses 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 epidermal nature of cultured cells was assessed by immunocytochemical staining of anticytokeratin (AE1/AE3; Boehringer Mannheim). Chromosome preparations 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.
2× SSC) and pepsin solution (12 3 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 solution (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 Red, Cy5, and Cy5.5). The probe (SKY mixture) was denatured at 75°C for 7 min and incubated at 37°C for 1 h. The hybridization solution was applied to the denatured metaphases and incubated for 2 days at 37°C. Posthybridization washes were performed in 50% formamide and 2× SSC (three times for 5 min), 1× SSC (twice for 5 min) at 45°C, and in 4× SSC and 0.1% Tween 20 for 2 min at room temperature. Detection of biotinylated and digoxigenin-labeled probes was performed with avidin Cy5 and goat anti-mouse antibody conjugated to Cy5.5. Metaphase preparations were stained with DAPI solution (150 ng/ml DAPI in 2× SSC) 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 image analysis software (24).

CGH. CGH was also performed on case S48 as described previously (22). Briefly, whole genomic DNA was isolated from the primary culture as well as from established cell lines according to standard procedures and labeled with biotin-16-dUTP (Boehringer Mannheim). Normal female reference DNA was isolated from the peripheral lymphocytes of a healthy donor and labeled with digoxigenin-11-dUTP (Boehringer Mannheim). After hybridization to the normal metaphase spreads of a healthy donor, labeled DNAs were detected with streptavidin Cy2 and antidigoxigenin/anti-mouse Cy3 conjugates. Slides were counterstained with DAPI (0.1 μg/ml in 2× SSC) for karyotyping and mounted in antifade solution. For CGH analysis, at least 10 metaphases were imaged and karyotyped after visualization with a Zeiss Axioplan 2 fluorescence microscope equipped with filter sets (single-band excitation filters) for DAPI, FITC, and tetramethylrhodamine isothiocyanate. Averaged profiles were generated by a CGH analysis software (MetaSystems, Altlussheim, Germany) from at least 10–15 homologous chromosomes and interpreted according to published criteria (25, 26).

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, S81, S95, S99, S125, S175, and S206; Tables 1–4). Two cases (S9 and S48) exhibited very complex structurally 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,del(16)(q13;qter), der(1;2)(p22;q34), t(3;14)(p14;q24), t(4;11)(q31;q23), del(5;7)(q23;p15), del(6;11)(q22;p11), del(7;9;15), der(9;13). CGH revealed chromosomal gains on chromosomes 1p34, 1q21, 2p11.1–pter, 2q11.2–q13 (high-level amplification), 3q26.2–
Tumors, a type of tumor that is normally rare in childhood. This is the first extensive cytogenetic study of childhood thyroid somal changes was also studied by SKY and CGH. To our knowledge, investigated by G-banding. One case exhibiting multiple chromosome abnormalities in Belarussian tumors and in secondary tumors affected by translocations as well as by chromosomal gains and losses.

Pooled results of an overall breakpoint analysis of structural chromosomal 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, 6q, 12q, and 13q were found in Belarussian tumors and secondary tumors after radiotherapy.

DISCUSSION

Karyotype abnormalities in childhood thyroid tumors from Belarus and from 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). Some cases (S81, S95, S99, and S125) exhibited high frequencies of damaged chromosomes that might reflect exposure to high doses of radioiodine. This is in good agreement with our earlier chromosome painting study without breakpoint analysis (20) that also revealed high translocation frequencies in a subset of Belarusian 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 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 in papillary thyroid tumors from adults without a radiation history (Fig. 2; Refs. 7, 8, 12, and 15–18). In particular, aberrations on 1q, 9q, and 10q (10q11 and 10q26) novel breakpoints on chromosomes 4q, 5q, 6q, 12q, 13q, and 14q were found in Belarusian tumors and secondary tumors after radiotherapy.

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

<table>
<thead>
<tr>
<th>Case</th>
<th>No. of metaphases studied</th>
<th>Total</th>
<th>Abnormal</th>
<th>Tumor classification</th>
<th>NO. of chromosomal aberrations</th>
<th>Pathological diagnosis</th>
<th>No. of metaphases</th>
<th>Tumor classification</th>
<th>NO. of chromosomal aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>S46</td>
<td>10/m</td>
<td>10</td>
<td>5</td>
<td>PTC</td>
<td>1</td>
<td>PTN2N2M1</td>
<td>10</td>
<td>High</td>
<td>1</td>
</tr>
<tr>
<td>S57</td>
<td>15/m</td>
<td>15</td>
<td>10</td>
<td>PTC</td>
<td>2</td>
<td>PTN2N2M1</td>
<td>15</td>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td>S75</td>
<td>13/f</td>
<td>13</td>
<td>12</td>
<td>PTC</td>
<td>1</td>
<td>PTN2N2M1</td>
<td>13</td>
<td>High</td>
<td>1</td>
</tr>
<tr>
<td>S81</td>
<td>9/m</td>
<td>9</td>
<td>8</td>
<td>PTC</td>
<td>1</td>
<td>PTN2N2M1</td>
<td>9</td>
<td>High</td>
<td>1</td>
</tr>
<tr>
<td>S95</td>
<td>14/m</td>
<td>14</td>
<td>13</td>
<td>PTC</td>
<td>1</td>
<td>PTN2N2M1</td>
<td>14</td>
<td>High</td>
<td>1</td>
</tr>
<tr>
<td>S99</td>
<td>10/f</td>
<td>10</td>
<td>9</td>
<td>PTC</td>
<td>1</td>
<td>PTN2N2M1</td>
<td>10</td>
<td>High</td>
<td>1</td>
</tr>
<tr>
<td>S124</td>
<td>8/m</td>
<td>8</td>
<td>7</td>
<td>PTC</td>
<td>1</td>
<td>PTN2N2M1</td>
<td>8</td>
<td>Low</td>
<td>1</td>
</tr>
<tr>
<td>S125</td>
<td>9/m</td>
<td>9</td>
<td>8</td>
<td>PTC</td>
<td>2</td>
<td>PTN2N2M1</td>
<td>9</td>
<td>Low</td>
<td>2</td>
</tr>
<tr>
<td>S135</td>
<td>9/f</td>
<td>9</td>
<td>8</td>
<td>PTC</td>
<td>1</td>
<td>PTN2N2M1</td>
<td>9</td>
<td>Low</td>
<td>1</td>
</tr>
<tr>
<td>S181</td>
<td>10/f</td>
<td>10</td>
<td>9</td>
<td>PTC</td>
<td>1</td>
<td>PTN2N2M1</td>
<td>10</td>
<td>Low</td>
<td>1</td>
</tr>
</tbody>
</table>

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

Table 4 Non-clonal structural chromosome aberrations in secondary thyroid tumors after therapeutic irradiation

<table>
<thead>
<tr>
<th>Case</th>
<th>No. of metaphases studied</th>
<th>Total</th>
<th>Abnormal</th>
<th>Tumor classification</th>
<th>NO. of chromosomal aberrations</th>
<th>Pathological diagnosis</th>
<th>No. of metaphases</th>
<th>Tumor classification</th>
<th>NO. of chromosomal aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>S9</td>
<td>53/m</td>
<td>53</td>
<td>49</td>
<td>Adenoma</td>
<td>2</td>
<td>PTN2N2M1</td>
<td>53</td>
<td>Low</td>
<td>2</td>
</tr>
<tr>
<td>S43</td>
<td>66/f</td>
<td>66</td>
<td>62</td>
<td>Struma</td>
<td>6</td>
<td>PTN2N2M1</td>
<td>66</td>
<td>High</td>
<td>6</td>
</tr>
<tr>
<td>S55</td>
<td>82/f</td>
<td>82</td>
<td>79</td>
<td>Basedow</td>
<td>6</td>
<td>PTN2N2M1</td>
<td>82</td>
<td>Moderate</td>
<td>6</td>
</tr>
<tr>
<td>S65</td>
<td>59/m</td>
<td>59</td>
<td>57</td>
<td>Medullary carcinoma</td>
<td>6</td>
<td>PTN2N2M1</td>
<td>59</td>
<td>High</td>
<td>6</td>
</tr>
<tr>
<td>S76</td>
<td>28/f</td>
<td>28</td>
<td>24</td>
<td>EX</td>
<td>4</td>
<td>Adenoma</td>
<td>28</td>
<td>Low</td>
<td>4</td>
</tr>
<tr>
<td>S106</td>
<td>56/f</td>
<td>56</td>
<td>52</td>
<td>EX</td>
<td>17</td>
<td>Adenoma</td>
<td>56</td>
<td>Low</td>
<td>17</td>
</tr>
<tr>
<td>S121</td>
<td>66/f</td>
<td>66</td>
<td>62</td>
<td>EX</td>
<td>63</td>
<td>Adenoma</td>
<td>66</td>
<td>Low</td>
<td>63</td>
</tr>
</tbody>
</table>

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

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


Cytogenetic Changes in Radiation-induced Tumors of the Thyroid

Horst Zitzelsberger, Lars Lehmann, Ludwig Hieber, et al.

*Cancer Res* 1999;59:135-140.