Comparative Genomic Instabilities of Thyroid and Colon Cancers

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**Objectives:** To assess the forms and extent of genomic instability in thyroid cancers and colorectal neoplasms and to determine if such measurements could explain the generally excellent prognosis of thyroid malignant neoplasms compared with colon carcinoma.

**Design:** Tumor genome analyses. Genomic instability was measured by the following 4 methods, listed in ascending order based on the size of events detected: inter-simple sequence repeat polymerase chain reaction (ISSR-PCR), fractional allelic loss (FAL) analysis, array-based comparative genomic hybridization (aCGH), and spectral karyotyping (SKY).

**Results:** The genomic instability index of 32 thyroid carcinomas, 59 colon carcinomas, and 11 colon polyps was determined by ISSR-PCR; no difference was seen among the 3 groups by this method. Fractional allelic loss rates were comparable in thyroid cancers and colon polyps and lower than FAL rates in colorectal cancers. Indolent papillary thyroid carcinomas were essentially diploid with no large-scale alterations in chromosome number or structure when evaluated by aCGH or SKY. In anaplastic thyroid cancers, aCGH revealed abundant chromosome alterations. Colorectal carcinomas showed extensive copy number changes and chromosomal rearrangements when analyzed by aCGH and SKY.

**Conclusions:** Genomic alterations in papillary thyroid carcinoma, such as in benign colon polyps, are principally smaller events detected by ISSR-PCR. With the more aggressive tumor types (ie, anaplastic thyroid and colorectal carcinomas), larger events detected by FAL analysis, aCGH, and SKY were revealed. We hypothesize that mutations caused by smaller genomic alterations enable thyroid cells to achieve a minimal malignant phenotype. Mutations for aggressive biological behavior appear with larger genomic events.

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in 2006. The majority of these colorectal cancers will have progressed through a benign polyp stage before achieving malignancy.

In an effort to determine the molecular basis for their very different biological behavior, we compared the patterns of genomic instability previously reported for colorectal carcinoma with those of thyroid cancers. Genomic instability was detected by bacterial artificial chromosome (BAC) array-based comparative genomic hybridization (aCGH), spectral karyotyping (SKY), loss of heterozygosity (LOH), and inter–simple sequence repeat polymerase chain reaction (ISSR-PCR) analyses. Genomic instability as measured by ISSR-PCR has been previously reported.

### METHODS

#### PATIENTS, TUMOR SPECIMENS, AND DNA PREPARATION

The 2 study populations, 59 patients with colorectal cancer and 33 patients with thyroid cancer, are described in detail elsewhere. Specimens were procured under the supervision of the institutional review board of the Roswell Park Cancer Institute, Buffalo, NY, and informed consent for participation in this study was obtained from all patients preoperatively. Histologically normal and tumor tissue from the same patient were procured immediately after surgery. Hematoxylin-eosin–stained sections of paraffin-embedded, neutral-buffered, formalin-fixed (10% formalin by volume in water; pH 7.4) specimens were reviewed by a pathologist to confirm the histologic features of the procured tissue. The tumor and normal tissue were either cultured, as described in the “Spectral Karyotyping” subsection, or stored at −70°C for subsequent DNA extraction for all other genomic assays. DNA extraction from the frozen tumor and normal tissue specimens was carried out as previously described.

#### INTER–SIMPLE SEQUENCE REPEAT PCR

The ISSR-PCR analyses were performed as previously described. Genomic instability index is the percentage of altered PCR products amplified from an individual’s tumor DNA compared with the PCR products of that individual’s normal DNA. This method detects alterations that have occurred in normal sequences between 2 microsatellites and is totally distinct from microsatellite instability.

#### LOH ASSAYS

The LOH assays were performed on 26 papillary thyroid cancers as previously described. Primers for all 22 autosomes are listed in Table 1. Fractional allelic loss (FAL) was calculated by determining the total fraction of all informative markers for a tumor DNA that showed LOH.

#### BAC ARRAY-BASED CGH

The details of BAC aCGH were as previously described by Cowl and Nowak. Briefly, DNA isolated from tumor tissues and a sex-mismatched pool of 15 normal DNA samples were separately labeled with either Cy3 or Cy5 fluorescent tags, mixed, and competitively hybridized to an array of over 6500 immobilized BAC clone targets. The ratio of red (Cy5) to green (Cy3) signal was quantified and plotted graphically to reveal amplifications and deletions within the tumor genome. Array-based CGH was performed on 16 papillary thyroid carcinomas and 4 anaplastic thyroid carcinomas.

### SPECTRAL KARYOTYPING

Short-term primary cultures of 7 papillary thyroid carcinomas were established according to the method of Matsui et al.
Table 2. Genomic Instability Profiles of Thyroid and Colorectal Neoplasms

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>ISSR-PCR*</th>
<th>FAL Analysis†</th>
<th>aCGH‡</th>
<th>SKY§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>+++</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>+++</td>
<td>Yes</td>
<td>+++</td>
<td>ND</td>
</tr>
<tr>
<td>Colon/rectum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Adenoma</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: aCGH, array-based comparative genomic hybridization; FAL, fractional allelic loss; ISSR-PCR, inter–simple sequence repeat polymerase chain reaction; ND, not determined; SKY, spectral karyotyping.

*Genomic instability index values of 0 to 0.2 (−); greater than 0.2 to 1 (+); greater than 1 to 2 (++); greater than 2 (+++).
†Genomic instability index values of 0 (−); 0.01 to 0.02 (+); greater than 0.02 to 0.08 (++); greater than 0.08 (+++).
‡Genomic instability index values of 0 to 1 (−); 2 to 4 (+); 5 to 10 (++); greater than 10 (+++).
§No aneuploidy or translocation (−); homogeneous aneuploidy or translocation (+); heterogeneous aneuploidy OR translocations (++); and heterogeneous aneuploidy AND translocations (+++).
||Based on Kitamura et al.14

RESULTS

Papillary thyroid cancer exhibits moderate clinical behavior compared with other malignant neoplasms such as colorectal carcinoma or anaplastic thyroid cancer; 10-year survival occurs in greater than 90% of patients with a diagnosis of having cancer.13 We investigated if the basis of this difference in clinical behavior might be attributed to the types or degree of genomic instability detected in these tumors. In several previous studies, we have used the genome sampling technique of ISSR-PCR to quantify one form of genomic instability in benign and malignant thyroid lesions and colorectal cancers and polyps.7,8 In addition, we have examined other forms of genomic instability in colorectal and colorectal cancers and polyps.9,10 In at least 10 metaphases were examined for each tumor specimen.

Genomic instability as measured by ISSR-PCR has been found to be an early event in colorectal carcinogenesis.9,14 Sequence analysis of altered ISSR-PCR products generated from tumor DNA has revealed that this technique preferentially detects small alterations in the genome.15 The mean genomic instability index estimate of genomic damage for 59 colorectal carcinomas was nearly identical to that measured in 7 adenomatous polyps (3.9% vs 4.1%).9 Our 2 subsequent analyses of papillary, follicular, and anaplastic thyroid cancers using ISSR-PCR revealed mean genomic instability index values of 2.9% and 3.1%9,10. These levels of genomic damage were not statistically different from those seen in colorectal carcinomas or polyps. Furthermore, the genomic instability index of the 1 anaplastic carcinoma available for study was 3.0%, suggesting that a large increase in this form of genomic instability is not responsible for the evolution to the more aggressive tumor type. These data are summarized in Table 2.

Our published study of allelic loss in colorectal cancers and polyps demonstrated a greater than 3-fold higher fraction of markers in carcinomas compared with adenomas; FAL rates were 0.095 in malignant neoplasms vs 0.03 in the benign lesions.1 Allelotyping generally detects allelic loss events of moderate size ranging from less than a megabase to entire chromosomes. The lower frequency of LOH in polyps indicates that these are events more closely associated with the later stages of tumor progression. Using a panel of 21 markers that map to 18 of the autosomal chromosomes, we interrogated the LOH incidence in 26 papillary thyroid cancers (Table 1). Fewer than half of the markers tested demonstrated 1 or more allelic losses. Loss of heterozygosity was detectable in 52% of the tumors, ranging from 0 to 0.154, with a mean FAL of 0.035 (range for colorectal cancers, 0 to 0.450). The mean level of FAL in papillary thyroid cancer was comparable to that observed in benign colon adenomas, far below levels seen in colorectal malignancies.

Bacterial artifical chromosome aCGH is a high-resolution screening technique that assesses DNA copy number aberrations in tumors. An analysis of 33 colorectal carcinomas revealed numerous gains and losses on nearly all chromosomes.3 As might be expected, polyps had significantly fewer chromosomal copy number aberrations, indicating that this form of instability generally occurs later in colon tumor progression.14 Interestingly, papillary carcinomas analyzed by aCGH were again found to be more similar to colon polyps than to carcinomas. The aCGH profiles for these cancers showed no significant deviation from normal diploid DNA (Figure 1). Occasional spikes were observed in one tumor or another, but no consistent alternations in copy number were observed. However, with the evolution to the more aggressive anaplastic thyroid tumor, aCGH detected amplifications and deletions on sev-
eral chromosomes. Most alterations were only found in a single tumor's DNA and may represent random DNA damage. The deletions and amplifications on the p and q arms, respectively, of chromosome 8 in Figure 1 were detected in 2 of the 4 anaplastic tumors.

To assess levels of genomic instability at the chromosomal level, 7 papillary thyroid cancer primary cultures were subjected to SKY. At least 10 metaphases were scored per tumor. All cells observed in all 7 tumors were scored as diploid with no detectable structural changes. A representative thyroid tumor cell is depicted in Figure 2A. Colon tumors cells were aneuploid, frequently pseudo-tetraploid, and contained numerous translocations (Figure 2B). Each panel in Figure 2B contains 1 or more altered chromosomes obtained from 7 different cells from the same colon tumor, indicating that colorectal tumors are highly heterogeneous. Anaplastic thyroid tumors were not available for primary cell culture.

The data summarizing all 4 measurements of genomic instability in papillary thyroid, anaplastic thyroid, and colorectal carcinomas, as well as colon adenomas, are given in Table 2. While all of these tumors possess ISSR-PCR–detected instability, those tumors that are associated with better prognoses (ie, papillary thyroid cancer and polyps) lack high-level genomic instability as measured by the remaining methods in Table 2.

Figure 1. Array-based comparative genomic hybridization of colorectal, papillary thyroid, and anaplastic thyroid carcinomas. Comparative genomic hybridization did not reveal gains or losses in indolent papillary thyroid carcinomas, while aggressive colorectal cancers and anaplastic cancer exhibited significant copy number aberrations. Chromosome position is shown on the x-axis; the vertical line indicates the position of the centromere. Representative results for chromosomes 7 (chr7) and 8 (chr8) are shown.

COMMENT

Genomic instability, generally accepted as a facilitator of solid tumor progression, occurs in 3 general forms, microsatellite instability, aneuploidy, and intrachromosomal instability. While much has been learned about the underlying mechanisms of microsatellite instability and aneuploidy, less is known of the molecular basis of intrachromosomal instability. Indeed, it now appears that intrachromosomal instability can be subdivided into several independent forms, each presumably with its own molecular origin, that are apparent when different assay methods are used.

In this study, we have compared the genomic instabilities detected in thyroid carcinomas with those observed in colorectal cancers and adenomas to determine if differences in their instability profiles were related to the distinct clinical behavior of the 2 cancers. Four methods were used to measure diverse genomic instabilities that result in genome alterations ranging in size from a few base pairs to whole chromosomes.

Our results demonstrate that thyroid cancers possess levels of genomic instability as measured by ISSR-PCR that are equivalent to those detected in both malignant and benign lesions of the colon or rectum. In addition, ISSR instability in anaplastic carcinoma was no greater than in papillary cancer. Therefore, this form of instability is not likely to be responsible for the evolution of aggressive behavior observed in the anaplastic carcinoma.

A meta-analysis of published studies of LOH in thyroid cancer performed by Ward et al17(p525) revealed that "papillary carcinomas had exceedingly low rates of LOH." In 2 separate genome-wide allelotyping studies, Vogelstein et al18 and Califano et al19 confirm that FAL levels (defined in both studies as the fraction of chromosomal

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arms lost) in papillary thyroid cancers are far lower than those of colorectal cancer. In addition, anaplastic thyroid cancer has been shown to exhibit numerous LOH events on several different chromosomes.\textsuperscript{16}

We were unable to find any reports of aCGH analysis of thyroid malignant neoplasms for comparison with our data. Our aCGH analysis detected few gains or losses in papillary thyroid carcinoma relative to the anaplastic form of the disease or to colon cancer. In addition, we did not detect any consistent changes. Several investigators have used metaphase-based CGH to analyze thyroid cancers. Bauer et al\textsuperscript{20} reported that only 4 of 15 papillary thyroid cancers exhibited less than 4 aberrations per tumor, with the remaining 11 having normal diploid karyotypes. Wreesmann et al\textsuperscript{21} found few gains or losses in 15 well-differentiated papillary thyroid cancers and numerous alterations in 27 poorly differentiated or anaplastic carcinomas. In a subsequent report from this group, higher numbers of gains and losses were reported in follicular variant of papillary carcinoma, but very few alterations were detected in classic papillary cancers.\textsuperscript{22} Miura et al\textsuperscript{23} demonstrated several copy number aberrations in anaplastic tumors and an anaplastic cell line, but only 2 copy number aberrations in the papillary cell line they evalu-
Early Events: "Small"
- Amplification
- Deletion

Later Events: "Large"
- Amplification
- Deletion
- Uniparentalism

Figure 3. Evolution of aggressive tumors is aided by multiple forms of genomic instability. Multiple forms of genomic instability arise sequentially during tumor progression. Early events, detected by inter–simple sequence repeat polymerase chain reaction, are copious but small. Many mutations remain silent and evolution to relatively "benign" neoplasms is enabled. Later events, detected by allelotyping, array-based comparative genomic hybridization, or spectral karyotyping, are larger and unmask previously silent mutations. Selection for aggressive disease occurs.

The earlier wave of genomic damage consists of thousands of small genome alterations that are readily detected by ISSR-PCR, not as readily detected by allelotyping, and rarely detected by assays such as aCGH or SKY (Figure 3A).

Because these events are small, the probability of hitting both copies of a particular sequence is low and the effects of some mutations remain masked. Those tumors in which this type of instability predominates tend to be relatively benign (ie, adenomas and papillary thyroid carcinomas). Subsequent waves of genomic instability tend to cause larger damage to the genome in forms such as deletions, amplifications, and uniparental conversion of some chromosomes, which can be revealed by allelotyping, SKY, and aCGH.

With the onset of large event–causing instabilities, the probability of revealing a previously silent mutation increases. Unmasked mutations are now able to contribute to the evolution of the tumor to more aggressive disease.

The tumors that exhibit multiple forms of genomic instability are clinically more aggressive tumors such as colorectal cancer and anaplastic thyroid cancer. The combination of these multiple forms of instability enable normal cells to evolve through benign neoplasms to highly aggressive malignant neoplasms.

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Author Contributions: Drs Stoler and Anderson had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Stoler, Nowak, Loree, and Anderson. Acquisition of data: Stoler, Nowak, Matsui, Wiseman, Chen, Dutt, Bartos, Loree, Rigual, Hicks, and Sait. Analysis and interpretation of data: Stoler, Nowak, Wiseman, Bartos, Loree, Rigual, and Anderson. Drafting of the manuscript: Stoler and Hicks. Critical revision of the manuscript for important intellectual content: Nowak, Matsui, Wiseman, Chen, Dutt, Bartos, Loree, Rigual, Sait, and Anderson. Statistical analysis: Bartos. Obtained funding: Loree and Anderson. Administrative, technical, and material support: Stoler, Nowak, Matsui, Wiseman, Chen, Dutt, Bartos, Loree, Rigual, Hicks, Sait, and Anderson. Study supervision: Anderson.

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REFERENCES


