Nonrandom Chromosomal Abnormalities in Lymphocyte Cultures of Individuals with Colorectal Polyps and of Asymptomatic Relatives of Patients with Colorectal Cancer or Polyps

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Abstract

We studied chromosomal alterations in the peripheral blood lymphocytes of 10 individuals with colorectal polyps and 10 asymptomatic first-degree relatives of patients with colon cancer or colorectal polyps. The analysis was performed on T-lymphocytes using short term blood cultures and on B-lymphocytes by establishing lymphoblastoid cell lines by Epstein-Barr virus transformation. Chromosomal changes were not common in T- and B-lymphocytes. Chromosomes 1 and 5 were most frequently involved in numerical or structural changes in the patients with polyps as well as in the asymptomatic relatives. These alterations were observed in either the T-lymphocytes or the B-lymphocytes but rarely in both, thus accentuating the importance of studying both the cultures concurrently. Chromosome 5, which is known to play an important role in the development of adenomatous polyps, was found to be involved in 6 (60%) of 10 patients with polyps and 4 (40%) of 10 asymptomatic relatives. These findings show that lymphocytic chromosomal analysis can aid in identifying individuals who are genetically susceptible and are at a higher risk of developing colorectal cancer. Because lymphocytic chromosomal analysis is relatively simple and inexpensive, we expect that it will be very useful in screening asymptomatic individuals who are at a higher risk due to inherited or environmental factors.

Introduction

Adenomatous polyps, which form discrete noninvasive intramucosal tumors that project into the bowel lumen, are examples of dysplastic colon epithelium that often progress to carcinomas (1). FAP, also known as adenomatous polyposis coli or familial polyposis coli, is an inherited autosomal dominant condition with a virtually 100% risk of malignant transformation. It has recently been proposed that FAP is not just a colonic disease, but that it can be genuinely designated as a genetically determined growth disorder that governs the development of benign and malignant lesions in different organs of the body (2). The genetic changes that occur in this condition may be present in a small number of somatic cells which could serve as a fertile ground in which to investigate chromosomal defects in individuals with polyps.

The localization of the FAP gene to chromosome 5 near bands q21–22 (3, 4), owes its discovery to the report regarding deletion in chromosome 5q in the lymphocytes of a 42-year-old white male patient with Gardner’s syndrome and mental retardation (5). Thus, FAP is the best example of lymphocytic analysis revealing the specific chromosomal alterations related to a particular disease (6). Preliminary results from our laboratory have indicated that lymphocytic analysis of individuals with polyps can help identify those at a higher risk of developing colorectal cancers (7). Peripheral blood lymphocytes of individuals with polyps revealed chromosome 5 anomalies in more than 50% of the cases investigated. Hence, we thought it worthwhile to further the investigation by exploring whether the chromosomal changes observed are confined to T-lymphocytes or whether these somatic-cell aberrations are also present in B-lymphocyte cultures of these individuals. Also, if a chromosomal aberration is present in somatic cells but at a frequency lower than 1%, it might escape detection by conventional T-lymphocyte analysis, but lymphoblastoid cell culture would provide suitable conditions for such cells to grow and thus help in drawing out this information. For this purpose, we established LCLs by Epstein-Barr virus transformation and analyzed their chromosomes.

First-degree relatives of patients with apparently sporadic colorectal cancer have been shown to have an increased incidence of adenomas and carcinomas. More recently, pedigree studies have suggested that an inherited predisposition may be responsible for most colorectal cancers (8). Hence, in addition to examining patients with polyps, we chose to study the asymptomatic relatives of some patients with colorectal cancer or polyps to determine...
whether they harbored any chromosomal changes in their T- and B-lymphocytes analogous to those found in the patients with polyps.

Materials and Methods
Peripheral blood samples were collected in heparinized vials from 10 untreated patients with colorectal polyps and 10 asymptomatic first-degree relatives of patients with colorectal cancer or polyps after procuring their written informed consent. Lymphocyte cultures were grown from 1 ml of whole blood in 9 ml of RPMI 1640 with folic acid (JRM biosciences, Lenexa, KS) supplemented with 20% fetal bovine serum (Sigma Chemical Co., St. Louis, MO), 2 mmol/l-glutamine, 50 units/ml penicillin, 100 μg/ml streptomycin, and 1.3% phytohemagglutinin (Wellcome Research Laboratories, Research Triangle Park, NC). The cultures were incubated at 37°C for 72 h. LCLs were established from mononuclear cell separated from whole blood with Sepacell-MN continuous density gradients (Sepacell Corporation, Oklahoma City, OK). The separated cells were added to equal volumes of RPMI 1640 containing 20% fetal bovine serum and filtered supernatant from an Epstein-Barr virus infected marmoset cell culture (B95-8). The cells were maintained in tightly capped T25 tissue-culture flasks in which they began transformation within approximately 30 days. Cytogenetic analyses were carried out at the first harvest approximately 45–50 days after initiation of cultures. For further analysis, the cells were frozen at −195°C.

For karyotyping, the cells were harvested after colcemid addition at a final concentration of 0.04 μg/ml, hypotonic treatment with 0.06 M KCl for 25 min, and aceto-methanol (1:3) fixation. Slides were prepared by a standard air-drying protocol and allowed to age before subjecting them to trypsin treatment for Giemsa banding (9). The slides were coded before cytogenetic analysis. At least 10 metaphases from each blood culture, and 50 metaphases from each of the LCLs were screened for chromosomal anomalies using Genetiscan (PSI, Houston, TX). Abnormal metaphases were karyotyped.

Results
Table 1 lists the clinical characteristics of the patients and asymptomatic individuals selected for this study. The numerical and structural abnormalities observed in the analysis of T- and B-lymphocytes are detailed in Tables 2 and 3. The two cultures from each individual did not have the same structural and numerical changes. It was interesting to note that chromosome 5 was the chromosome most frequently involved in the rearrangements. Six of the 10 polyps in the asymptomatic patients manifested chromosome 5 anomalies. In three patients these anomalies were observed only in the B-lymphocytes and in two patients only in the T-lymphocytes. In only one patient, chromosome 5 was found to be rearranged in both cultures (BL 4251/SP 1149). The alterations in chromosome 5 were observed in four asymptomatic individuals three times in only the B-lymphocytes and once in both the T- and B-lymphocytes. Chromosome 1 was the second most common chromosome to be involved in aberrations. Clonality of a cell was rare among the T-lymphocytes and most of the chromosomal aberrations were exclusive events. Only SP 2242 and SP 649 of the polyposis patients and SP 2064 of the asymptomatic individuals exhibited clonality.

Putting together the abnormalities observed in both the T- and B-lymphocytes of patients with polyps, chromosomes 5 and 1 were the most frequently involved in structural or numerical rearrangements, followed by chromosomes 12 and 7 (Fig. 1). Interestingly, a similar picture emerged on analyzing the T- and B-lymphocytes of the asymptomatic individuals. Chromosomes 5 and 1 were the most frequently rearranged structurally or numerically (Fig. 1). If we had analyzed only T-lymphocytes, the aberrations of chromosome 5 would have been found in only one (BL 4795/SP 2240) out of the 10 samples of the asymptomatic individuals, but when both T- and B-cells were scrutinized for chromosomal changes, 4 (40%) of 10 samples were found to contain aberrations of chromosome 5 which included 1 individual with break in chromosome 5 in both the T- and B-lymphocytes (BL 4795/SP 2240) (Table 3).

Discussion
The development of colorectal neoplasia is associated with progressive accumulation of multihit genetic changes. Recent studies suggest that colon cancer results from the aggregate effects of multiple genetic mutations which may be inherited (germline) or acquired (somatic) (10). There is evidence that adenomatous polyps are the precursors of carcinomas and that carcinomas develop at the sites of preexisting untreated adenomas (11). Family studies have revealed that there is an increased incidence of colorectal adenomas and carcinomas in the relatives of colorectal cancer probands, and it has also been suggested that predisposition to adenomatous polyps is a dominantly inherited trait (12, 13). This implies that genetic susceptibility is an important feature in the development of colorectal premalignant and malignant diseases. However, the exact inheritance pattern of colon-associated neoplasms is not known. The involvement of the FAP gene located on chromosome 5q in sporadic as well as familial colorectal cancers suggests that it has a bearing on the etiology of common colorectal cancer (14–17). Allelic loss has also been observed in premalignant and sporadic colorectal adenomas (18); hence, it is likely that alterations in the FAP gene occur early in the
Table 2  Chromosomal abnormalities observed in the T- and B-lymphocytes of patients with colorectal polyps

<table>
<thead>
<tr>
<th>Patient</th>
<th>T-lymphocytes</th>
<th>B-lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL 3764/SP 649</td>
<td>None</td>
<td>46,XY;[t(1;3)(q21;q27);in 7 cells]</td>
</tr>
<tr>
<td>BL 3818/SP 802</td>
<td>46,XX;[t(11;7)(p15;q26)]</td>
<td>47,XX,+4</td>
</tr>
<tr>
<td>BL 3821/SP 801</td>
<td>47,XX,+21</td>
<td>None</td>
</tr>
<tr>
<td>BL 4227/SP 119</td>
<td>46,XY;[t(15;10)(p13;q22)]</td>
<td>47,XY,+12,t(14;18)(q32;q21)</td>
</tr>
<tr>
<td>BL 4228/SP 1116</td>
<td>47,XX,+6</td>
<td>46,XX;[t(5;11)(p13;p14)]</td>
</tr>
<tr>
<td>BL 4245/SP 1148</td>
<td>46,XX;[t(1;7)(p32;p22)]</td>
<td>46,XX;[t(7;12)(q22;q21)]</td>
</tr>
<tr>
<td>BL 4251/SP 1149</td>
<td>46,XY;[t(5;34;q11)]</td>
<td>46,XX;[t(11;13)(q32;q14)]</td>
</tr>
<tr>
<td>BL 4524/SP 2094</td>
<td>46,XY;[d(9;11)]</td>
<td>45,XX,+16,–10,–21,d(6p23)</td>
</tr>
<tr>
<td>BL 4841/SP 2242</td>
<td>45,XX;[t(1;11)(q23;q23),</td>
<td>46,XX;[t(5;19)(q13;q12)]</td>
</tr>
<tr>
<td></td>
<td>d(7);t(7;9)(q32;q32) =pter;p22)]</td>
<td>45,XX,+12</td>
</tr>
<tr>
<td>BL 5021/SP 2346</td>
<td>47,XY;[t(5;19)(q13;q12)]</td>
<td>46,XY,+16,–9</td>
</tr>
</tbody>
</table>

*Every chromosomal aberration was observed in a single cell except where indicated. BL = T lymphocytes; SP = B lymphocytes.

development of colorectal cancer. The genetic defect that confers predisposition to FAP probably operates at the stage of conversion of normal mucosal cells to adenomatous polyps, and this occurs in somatic cells.

Peripheral blood lymphocytes represent normal cells, i.e., they are not the target tissue in FAP. Peripheral blood offers ample metaphase cells amenable for analysis and the advantage of noninvasive sample collection. An earlier report from our laboratory showed that chromosome 5 was involved in more than 50% of the peripheral blood samples of polyposis patients and that the alterations in chromosome 5 involved different breakpoints (7). The data were based on T-lymphocyte analysis only. In the investigation presented here, both T- and B-lymphocytes were analyzed. LCLs represent the B cells and provide suitable conditions for abnormal cells to multiply. This approach also allows one to store and retrieve samples for further cellular and molecular analyses should they be necessary. We found that T- and B-lymphocytes exhibited different chromosomal aberrations, which implies either that the abnormalities arose in different cells or that if they were stem cell aberrations, the frequency of each was not high enough (more than 1%) to appear in both of the paired samples. It is significant that chromosome 5 aberrations were equally frequent in T- and B-lymphocytes. No other chromosome except chromosome 1 exhibited as many changes as chromosome 5 did. The nonrandom and preferential involvement of chromosome 1 in most solid tumors, including colon cancers, has been reported earlier (19–21). Increased genetic instability among the lymphocytes of FAP patients has been reported by others (22); however, those studies examined only T-lymphocytes. Since B-lymphocytes also showed chromosome 1 and 5 alterations, it would be interesting to further analyze the susceptibility of these chromosomes to breakage by challenging the cells with a carcinogen. One patient (BL 4524/SP 2094) had (basal cell) skin cancer as well as adenomatous polyps. The LCL (SP 2094) of this patient manifested the aberration of chromosome 6 which has been implicated in melanoma (23). These results emphasize the need to analyze both cultures concurrently.

Alterations in chromosome 12 were also frequently observed in patients with polyps (Fig. 1; Table 2). The current progress in identifying the genetic changes in colorectal cancers has shown that tumorigenesis results from both the loss of growth suppressor genes on several chromosomes, including 5, 17, and 18, and also from the activation of a dominantly acting protooncogene on chromosome 12 (8, 18, 24). It is generally believed that chromosome 5 allele loss and K-ras mutations on chromosome 12 are early events. Chromosome 18 allele loss occurs predominantly in large...
### Table 1  Chromosomal abnormalities detected in the T- and B-lymphocytes of asymptomatic first-degree relatives of patients with colorectal polyps or colorectal cancer.

<table>
<thead>
<tr>
<th>Patient</th>
<th>T-lymphocytes</th>
<th>B-lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL 4409/SP 2064</td>
<td>45,XY,der(10;p14:10:p14:10)</td>
<td>46,XY,del(10;p14:10:p14:10)</td>
</tr>
<tr>
<td>BL 4411/SP 2058</td>
<td>46,X;+X(q22)</td>
<td>None</td>
</tr>
<tr>
<td>BL 4515/SP 2088</td>
<td>46,XY,der(1;31;p26;q21),t(4;14)(q12;q12)</td>
<td>46,XY,del(1;31;p26;q21),t(4;14)(q12;q12)</td>
</tr>
<tr>
<td>BL 4631/SP 2113</td>
<td>46,XY,del(12p12),t(4;13)(p12;q12)</td>
<td>None</td>
</tr>
<tr>
<td>BL 4795/SP 2240</td>
<td>46,XX,iso(7)(q11:q13)</td>
<td>45,XX,alt(5q13:q13)</td>
</tr>
<tr>
<td>BL 4992/SP 2330</td>
<td>Break 16 (q11)</td>
<td>46,XX,del(11q22:q23)</td>
</tr>
<tr>
<td>BL 5022/SP 2347</td>
<td>None</td>
<td>46,XX,del(11q11:q13)</td>
</tr>
<tr>
<td>BL 5023/SP 2348</td>
<td>None</td>
<td>45,XX,−19</td>
</tr>
<tr>
<td>BL 5025/SP 2332</td>
<td>None</td>
<td>45,XX,−5</td>
</tr>
<tr>
<td>BL 5240/SP 2441</td>
<td>47,XX,t(4;11q12;q14)</td>
<td>45,XX,−8,17t(14;p12:q22)</td>
</tr>
</tbody>
</table>

Every chromosomal change was observed in a single cell except where indicated. BL = T lymphocytes; SP = B lymphocytes.

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**Fig. 1.** Number of structural or numerical chromosomal rearrangements observed in T- and B-lymphocytes together of patients with colorectal polyps and in asymptomatic first-degree relatives of patients with colorectal cancer or polyps.
adenomas while chromosome 17 loss is usually restricted to carcinomas (8, 25, 26). However, Fearon and Vogelstein (27) emphasized that the net sum of genetic events, rather than the sequence of occurrence, determines the result. In this study of chromosomal aberrations in normal tissue, we observed that chromosome 17 was never involved in numerical or structural alterations among polyposis patients, although it was found to be rearranged in two of the asymptomatic family members. Aberrations in chromosome 18 were also infrequent, thus supporting the hypothesis that alterations in chromosomes 17 and 18 are late events in colon tumor progression.

The chromosomal alterations in the asymptomatic first-degree relatives of patients with colorectal cancer or polyps were very similar to those observed in polyposis patients. Family studies have shown that the first-degree relatives of colorectal cancer and adenoma patients have an increased risk of colorectal cancer and that familial factors are associated with the formation and not the progression of adenomas to carcinomas (8). Our unpublished data on the T lymphocytes of another 62 asymptomatic colorectal-cancer family members show involvement of chromosomes 5, 12, 17, and 18 in 9 (14.5%), 15 (24.2%), 5 (8.1%), and 6 (9.7%) individuals, respectively. The results of our chromosomal analysis of T- and B-lymphocytes of the asymptomatic family members emphasizes that clinically normal individuals do exhibit specific aberrations in chromosomes responsible for the development of polyps. Because genetic make-up plays an important role in colon carcinogenesis, the asymptomatic relatives of colon cancer patients may be more prone to develop colon cancer. Our findings suggest that a relatively noninvasive and inexpensive technique of analyzing lymphocytes can help in identifying individuals who might be more susceptible to develop premalignant defects. Long-term surveillance of the individuals with specific chromosomal alterations, however, would prove to be more meaningful.

Acknowledgments

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References

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