Deletion of 1p36 as a Primary Chromosomal Aberration in Intestinal Tumorigenesis

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ABSTRACT

Cytogenetic analysis of short-term cultures from benign intestinal tumors revealed clonal chromosomal aberrations in five colorectal adenomas, one adenoma of the papilla Vateri, and one hyperplastic polyp of the rectum. One adenoma had numerical aberrations only, but in all other tumors structural rearrangements were found that led to loss of genetic material from 1p. In three of the cases, the deletion was restricted to the 1p36 band; the rest had lost larger 1p segments. The rearrangement of chromosome 1 was the sole karyotypic anomaly in three adenomas, all with mild or moderate dysplasia, and in the hyperplastic polyp. Both adenomas that had additional aberrations beyond the 1p loss showed severe dysplasia. We conclude that cytogenetically detectable loss of genetic information from 1p36 is an early, seemingly primary, premalignant event in intestinal tumorigenesis. The fact that the adenomas with 1p− as the sole change showed only mild or moderate dysplasia and that the del(1p) was found also in the hyperplastic polyp suggests that this aberration is more related to the induction of hyperproliferation than to differentiation disturbances in the intestinal mucosa.

INTRODUCTION

Spontaneous carcinogenesis is generally understood to be a multistage process (1) which (according to the somatic mutation theory of cancer) is driven by the sequential acquisition by the tumor cells of nonrandom, sometimes highly specific, genomic alterations. Although some of these mutations are too small to be seen at the cytogenetic level, many are microscopically visible as structural or numerical chromosome anomalies (2). These acquired, tumor-associated, clonal chromosome aberrations can be broadly divided into primary abnormalities, which presumably play a causal role in the early neoplastic transformation of cells, and secondary abnormalities, which accrue later and are thought to be important in tumor progression (3–6).

Because carcinomas often harbor many chromosomal aberrations (2) at the time they are analyzed, it may be impossible to determine which are primary and which are secondary changes. One way to get around the problem is by investigating early lesions that have not yet become malignant; these would be expected to have only one or a few aberrations. In intestinal carcinogenesis, this means studying adenomas. Although a development via an adenomatous stage is probably not ubiquitous for colorectal carcinomas, especially not for ulceroinfiltrative tumors, most large bowel cancers do seem to arise from adenomas (7, 8). Another macroscopically detectable lesion that seems to have some relationship to carcinomas, albeit probably not as a direct forerunner (9), is the metaplastic or hyperplastic polyp. The hyperplastic epithelium of these polyps, unlike that of adenomas, is without dysplasia.

In this report, we describe the detection of abnormal karyotypes in seven benign intestinal tumors: six adenomas and one hyperplastic polyp. Structural rearrangements leading to loss of genetic material from the short arm of chromosome 1 were found in five of the adenomas and in the polyp. This suggests that del(1p) is an early event in intestinal tumorigenesis and that the change probably has more to do with the transition to a hyperproliferative growth pattern than with the loss of normal cellular differentiation.

MATERIALS AND METHODS

The material consists of a consecutive series of 13 clinically benign, >1-cm-diameter intestinal tumors. From each tumor, a sample of at least 0.5 cm was obtained at the time of surgery and processed for short-term culturing and cytogenetic analysis. The remaining tissue was totally embedded in paraffin and stained with hematoxylin and eosin. The section next to the tissue used for cytogenetic analysis was marked. The histological examination was undertaken without knowledge of the karyotypic characteristics of the tumors. Classification and grading were performed according to WHO recommendations (10), a system which in our hands has shown good reproducibility (11). The highest grade of dysplasia present in each tumor was noted as the result. A summary of the relevant clinical and pathologic data on the seven cytogenetically informative cases (see below) is given in Table 1.

The tumor samples intended for cytogenetic analysis were brought directly to the laboratory in sterile plastic tubes in a washing medium that consisted of Dulbecco’s modified Eagle’s medium/F12 (1:1) with added penicillin (200 IU/ml), streptomycin (0.4 mg/ml), gentamycin (200 µg/ml), and amphotericin B (2.5 µg/ml). The samples were then washed at least twice in fresh washing medium, transferred to sterile Petri dishes, minced with scissors, and enzymatically disaggregated for 4–5 h in collagenase type II (1300 units/ml; Worthington) in a humidified incubator (37°C) in 5% CO2 in air. The resulting suspension was spun down at 200 × g for 10 min, resuspended in washing medium, and spun down again. The pellet was resuspended in growth medium and plated out in 25-cm2 plastic flasks (Primaria; Falcon). The growth medium consisted of Dulbecco’s modified Eagle’s medium/F12 (1:1) with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, to which had been added 5% fetal bovine serum, glutamine (0.23 mg/ml), penicillin (100 IU/ml), streptomycin (0.2 mg/ml), gentamycin (100 µg/ml), amphotericin B (2.5 µg/ml), epidermal growth factor (10 ng/ml), hydrocortisone (0.5 µg/ml), cholera toxin (50 µg/ml), dibutyryl cyclic AMP (10 µm), and 1% ITS+ (Flow; final concentrations: insulin, 6.25 µg/ml; transferrin, 6.25 µg/ml; selenious acid, 6.25 µg/ml; bovine serum albumin, 1.25 mg/ml; linoleic acid, 5.35 µg/ml). When numerous cell clumps had attached (after 24–48 h) the surplus medium, which then always contained floating cells and cell clumps, was transferred to additional flasks, directly or after centrifugation and resuspension, in an attempt to establish more primary cultures. The growth medium was changed every 2 days. The cultural morphology was overwhelmingly epithelial in all cases.

After 5–10 days, colcemid was added for 4–6 h (0.06 µg/ml) or overnight (0.02 µg/ml), the cultures were harvested, and chromosome preparations were made. The preparations were incubated overnight in air and then for 3 h in 2× sodium saline citrate, (1 × SSC = 0.3 M NaCl and 0.03 M trisodium citrate) both at 60°C. After at least another 2 h of drying at room temperature, the chromosomes were banded using Wright’s stain. In the subsequent cytogenetic analysis, the clonality criteria and description of tumor karyotypes followed the recommendations of the International System for Human Cytogenetic Nomenclature, 1991 (12).
DELETION OF 1p36 IN INTESTINAL TUMORIGENESIS

Table I Summary of clinical, pathologic, and cytogenetic findings in the seven cases with karyotypic abnormalities

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/sex</th>
<th>Size (cm)</th>
<th>Site*</th>
<th>Histology*</th>
<th>Dysplasia</th>
<th>Karyotype*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55/F</td>
<td>3</td>
<td>C</td>
<td>TV</td>
<td>Moderate</td>
<td>47.XX,+5(6)</td>
</tr>
<tr>
<td>2</td>
<td>59/F</td>
<td>2</td>
<td>C</td>
<td>T</td>
<td>Mild</td>
<td>46.XX,del(1p36)inv(1p34q42)</td>
</tr>
<tr>
<td>3</td>
<td>61/F</td>
<td>3</td>
<td>R</td>
<td>TV</td>
<td>Severe</td>
<td>45.XX,del(1p34),inv(6q21q27),−18(10)</td>
</tr>
<tr>
<td>4</td>
<td>72/F</td>
<td>5</td>
<td>R</td>
<td>TV</td>
<td>Moderate</td>
<td>46.XX,del(1p36)</td>
</tr>
<tr>
<td>5</td>
<td>66/M</td>
<td>4</td>
<td>PV</td>
<td>TV</td>
<td>Severe</td>
<td>44.XY,add(1p13),−5,del(6q12q16),−21(13)</td>
</tr>
<tr>
<td>6</td>
<td>70/F</td>
<td>1</td>
<td>C</td>
<td>T</td>
<td>Mild</td>
<td>46.XX,del(1p36)</td>
</tr>
<tr>
<td>7</td>
<td>67/M</td>
<td>1</td>
<td>R</td>
<td>HP</td>
<td></td>
<td>46.XY,del(1p13)</td>
</tr>
</tbody>
</table>

* C, colon; R, rectum; PV, papilla Valeri.
* TV, tubular-villous; T, tubular; HP, hyperplastic polyp.
* Numbers in square brackets denote the number of mitoses in each clone.

RESULTS

Because of infection observed within 24 h in cultures from 3 samples and insufficient cell outgrowth and number of mitoses in the cultures from another 3 samples, only 7 of the 13 cases could be successfully karyotyped. All 7 tumors had clonal karyotypic abnormalities (Table 1). The modal chromosome number was always in the diploid range (diploid in cases 2, 4, 6, and 7; hyperdiploid in case 1; and hypodiploid in cases 3 and 5). Cells with a normal chromosome complement were found in addition to the abnormal mitoses in all tumors but case 2; in this patient, analysis of peripheral blood lymphocytes revealed a normal karyotype.

Numerical aberrations only were found in case 1, apparently in two cytogenetically unrelated clones. The remaining six tumors had structural rearrangements that involved chromosome 1 (Fig. 1) and led to loss of 1p material (straightforward deletions in five tumors, but also the addition of a block of unknown material to band 1p13 in case 5 must have led to 1p loss, i.e., of the segment 1p13→1pter). Whereas the aberrations of chromosome 1 were the only detectable changes in four tumors (cases 2, 4, 6, and 7), they were accompanied by additional abnormalities in cases 3 (Fig. 2) and 5.

DISCUSSION

Our finding of loss of material from the short arm of chromosome 1 in five of six successfully karyotyped intestinal adenomas (all but one colorectal) and in a rectal hyperplastic polyp strongly indicates that 1p deletions are much more common in colorectal adenomas than chance would allow. This is particularly so since the chromosome 1 aberration was the only change in four of the tumors, thus identifying del(1p) as the primary cytogenetic anomaly in at least this subset. The size of the deletion was not identical in all cases, but 1p36 was always lost. In three cases (2, 4, and 6 in Table 1; Fig. 1), the deletion seemed to be restricted to this band, which may be said to constitute a minimal common deleted segment in our series.

To the best of our knowledge, fewer than 30 intestinal adenomas with karyotypic abnormalities have been reported until now (2). No clearly nonrandom rearrangement has been detected; most tumors seem to have been characterized by variable numerical aberrations (13–15), in a manner rather similar to that of case 1 of the present series. Several of the adenoma karyotypes have been incompletely described. In spite of the uncertainties thus involved, rearrangements of 1p have been identified in three cases (14, 16, 17), in two of them leading to visible loss of chromosomal material (14, 17). Band 1p36 was part of the lost segment in one mildly dysplastic tubulovillous adenoma, which had del(1)(p32) as the only clonal change (17). The literature data therefore provide some support for our conclusion that the deletion of 1p36 is a primary chromosome aberration in colorectal adenomas. Why other investigators have found the deletion less often than we did remains uncertain. Until now, no loss of heterozygosity studies using 1p probes have been reported in large bowel adenomas, and so no molecular genetic evidence can be relied upon to temper the cytogenetic assessments of the relevant frequencies.

Microscopically visible loss of material from the short arm of chromosome 1 is detected in roughly 50% of all cytogenetically
abnormal colorectal adenocarcinomas (18–21), and molecular-genetic investigations have revealed losses in comparable, if not higher, percentages (22, 23). However, the changes leading to lp loss were generally supposed to be more characteristic of advanced, metastatic cancers than of early-stage tumors (23). In the light of the present findings, this supposition now appears to be in need of modification; del(lp) seems to precede malignancy-specific changes in a fair subset of colorectal neoplasms, although, as pointed out already by Foulds (1), any attempt to determine the preferred sequence of pathogenetic events should take into account that their eventual sum probably is more important than their temporal relationship. Nevertheless, a certain sequential order exists for the acquisition of genetic changes by budding tumor cells, also in colorectal carcinogenesis (24), and the del(lp) then seems to have its place as an early, if not the earliest, aberration. Of course, the available data do not give any information as to how likely an adenoma with lp− is to acquire the additional changes that will force it further down the road toward becoming an infiltrating cancer.

The conclusion that del(1)(p36) is an early change in intestinal mucosa tumorigenesis is also supported by a comparison between the histological and cytogenetic findings in our series. Of the four tumors which had karyotypic abnormalities restricted to chromosome 1, one was an adenoma with moderate dysplasia, two were adenomas with mild dysplasia, and one was the hyperplastic polyp, i.e., a lesion characterized by hyperplasia but without dysplasia. To these cases can be added the adenoma with del(1)(p32) as the sole karyotypic abnormality that was reported by Couturier-Turpin et al. (17), which was only mildly dysplastic. In contrast, the two adenomas with loss from lp as well as secondary aberrations both displayed severe dysplasia (Table 1; Fig. 2). The del(1)(p36) therefore gives the impression of being much more capable of stimulating proliferation than inhibiting differentiation. In a division of cancer-associated chromosomal breakpoints (and, by inference, the gene loci they correspond to) into those that are proliferation specific and those that are differentiation associated (25), the lp36 site appears to be of the former type. Such a role would also be in line with the seemingly non-tissue-specific nature of lp36 changes which, in addition to being found in intestinal tumors, are also detected in neoplasms as diverse as neuroblastoma, melanoma, breast and cervical carcinoma, and uterine leiomyoma (2).

How loss of genetic information from lp36 elicits a hyperplastic response is unknown; that, as well as the question of how much heterogeneity might exist among the various lp deletions in different tumor types, can be resolved only by molecular genetic, not cytogenetic, methods.

ACKNOWLEDGMENTS

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