Genetic Pathways and Genome-Wide Determinants of Clinical Outcome in Colorectal Cancer

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ABSTRACT

Various studies have suggested the existence of different pathways of tumor progression in colorectal cancer that associate with specific molecular, chromosomal, and clinicopathological features. We hypothesize that a comprehensive analysis of cumulated genomic damage in colorectal cancers would aid the characterization of different tumor progression pathways and identify the factors determining clinical outcome of tumors of each type. Genome-wide disruption was studied by DNA fingerprinting in a series of 129 sporadic colorectal carcinomas. These results, taken together with data for DNA ploidy, microsatellite instability, p53, and K-ras mutations and clinicopathological characteristics of the patients, have been used to classify colorectal carcinomas. The following five groups can be defined based on the type and level of cumulated genomic damage: (a) tumors with microsatellite instability, right location, and good prognosis; (b) diploid tumors lacking p53 mutations, left and right location, low subchromosomal damage, and bad prognosis; (c) diploid tumors with p53 mutations, left location, high levels of subchromosomal damage, and good prognosis; (d) high aneuploid tumors, p53 mutations, left location, high levels of numerical and structural chromosomal alterations, and bad prognosis; and finally (e) low aneuploid tumors, no p53 mutations, left and right location, low levels of structural chromosomal alterations, and good prognosis. We postulate that these groups represent alternative pathways of tumor progression, each with determinants of aggressiveness. This indicates a need for different prognostic assessments depending on which group the tumor belongs to.

INTRODUCTION

Colorectal cancers are characterized by multiple chromosomal abnormalities and appear as one of the best examples of the multistep nature of tumorigenesis. Tumor progression is an evolutionary process determined by two main factors: the generation of heterogeneity and the selection of variants most suited to survival, growth, and invasion (reviewed in Ref. 1). Although most of the data generated thus far fit within such framework (reviewed in Ref. 2), the underlying mechanisms that sustain tumor evolution are mostly unknown, with the exception of tumors with the microsatellite mutator phenotype (3). In these tumors, defective DNA repair machinery (4) results in the accumulation of extensive genomic damage, most of it in noncoding microsatellite DNA sequences (MSI). Eventually, mutations also occur in sequences located in the coding region of genes involved in molecular pathways controlling key processes in cell proliferation and differentiation (5). Subsequently, cell clones harboring advantageous genetic changes are selected for and expanded (2).

Because most colorectal cancers progress without MSI, alternative mechanisms have been postulated (6, 7). Chromosomal instability has been proposed as the agent responsible for the genomic disruption observed in the majority of colorectal cancer cells (6, 8). Although this is an attractive hypothesis, the nature of this instability (or instabilities) and the underlying mechanism(s) are still a matter of debate. As well as experimental and epidemiological evidences, there are mathematical models that support the concept that a few mutational events may be enough to initiate the cell’s transformation with no need of a mutator phenotype (9–12).

On the basis of the heterogeneous nature of the genetic and epigenetic alterations, taken together with distinctive morphological and biological features exhibited by tumors, alternative genetic pathways have been proposed in colorectal carcinogenesis (3, 7, 13–24), although the heterogeneous nature of the experimental approaches used to define the subtypes of colorectal tumors and the diversity of sample collections precludes the establishment of agreed models with potential applicability.

To add the identification of the genetic pathways of progression in colorectal tumorigenesis, we have explored the interrelationships between specific molecular alterations, genomic disruption profiles, and clinical features in a series of 129 carcinomas collected prospectively. Comprehensive approaches resolving chromosomal and subchromosomal alterations have been used to ascertain the type and degree of chromosomal instability. Multivariate correlates of molecular profiles have enabled us to classify colorectal carcinomas into five groups with distinctive features and determinants of clinical outcome. We also propose that the use of this classification may be useful to better reveal the factors governing the clinical outcome in each group.

MATERIALS AND METHODS

Patients and Samples

A total of 129 patients was included in this study based on the availability of high-quality DNA from paired normal and tumor tissue. These cases were part of a larger series (n = 151) of patients preoperatively diagnosed with colorectal cancer at the Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) and prospectively included in a study designed to evaluate the prognostic value of specific genetic alterations, as well as the estimation of overall genetic damage assessed by several techniques. Inclusion in the study did not influence the adjuvant treatment given. The study protocol was approved by the Ethics Committee. No chemotherapy or radiotherapy was given before surgery in these patients. Inclusion criteria and main characteristics of the tumors, as well as the results of different genetic analyses, were as reported previously (25, 26). Briefly, the most important characteristics of the 129 cases included in the study were: 73 males and 56 females; mean age 66 ± 12 years (range, 33–96 years). Forty tumors were located in the right colon and 89 in the left colon, including the rectum. The distribution of the carcinomas according to modified Dukes’ classification was 18 A and B1, 54 B2 and B3, and 40 C and 17 D. According lymph node invasion (N), 78 tumors were N0, 33 N1 (1–3 positive lymph nodes), and 18 N2–N3 (>3 positive lymph nodes). One hundred eight patients underwent radical surgical resection defined by the absence of macroscopic or microscopic remnant disease and not Dukes’ D (RO). At the last follow-up (January 1999), 72 patients were alive without disease, 6 dead without disease, 1 alive with disease, and 50 had died because of the disease. Mean follow-up was 65 ± 14 months (range, 19–85 months).
Quantification of Chromosomal and Subchromosomal Damage by AP-PCR

Generation of DNA Fingerprints. Genetic alterations at subchromosomal level were assessed using the DNA fingerprinting technique AP-PCR. Fingerprints were generated in three independent AP-PCR amplifications performed with different primers. Primers were selected based on reproducibility and pattern readability according to previous studies performed in a distinct set of samples (27, 28). Assay conditions of the three AP-PCR experiments have been described previously (27, 28). Reaction mix contained $^{32}$P-dATP to visualize the bands. PCR products were diluted (1:4) with 95% deionized formamide denaturing buffer, heated at 95°C for 3 min, and immediately cooled on ice. Three μl of the mixture were analyzed in a 6% polyacrylamide 8 m urea-denaturing sequencing gel run at 55 W from 3 to 6 h, depending on the primer. The gels were dried under vacuum at 85°C and exposed to X-ray film at room temperature without an intensifier screen for 2–4 days.

Band Definition and Scoring of Band Intensity Variations. Films were scanned, and the intensity of the bands was densitometrically measured using Phoretix 1D Advanced version 3 (Nonlinear Dynamics, Newcastle upon Tyne, United Kingdom). Band intensity values were normalized in each lane to compensate for differences in sample loading, labeling, and exposure. Fingerprints of paired normal and tumor tissues were always run next to each other and compared with determine band intensity differences (Fig. 1). The threshold of variability was defined from a set of reproducibility experiments and adjusted by band intensity. Changes of band intensity between normal and tumor tissue were considered significant when the difference, measured as a ratio, was above the 95% CI of reproducibility determinations (data not shown). Differences were scored as losses and gains to reflect decreases and increases of band intensity in the tumor in regard to the paired normal tissue.

Index of Genomic Damage. The GDF was calculated for each tumor as the number of bands with increases or decreases of intensity divided by the total number of bands visualized. GF and LF were also calculated considering both types of alterations separately.

![Image of fingerprints]

**Fig. 1.** AP-PCR fingerprints of a normal tumor DNA pair performed with each one of the primer sets. Illustrative examples of gains (up arrowheads) and losses (down arrowheads) are shown next to each lane.

RESULTS

Characterization of Genomic Damage by AP-PCR. AP-PCR products resolved in sequencing gels resulted in fingerprints composed of 40–60 bands that correspond to unique genome-wide distributed anonymous DNA sequences (Fig. 1). The results of the three AP-PCR experiments were considered jointly. Sixteen cases (12%) failed to yield reproducible fingerprints for either the normal or the tumor tissue DNA and were excluded from the AP-PCR analysis. The number of informative bands/case was 141 ± 27.

Three different indexes of genomic damage were obtained representing the rates of allelic losses LF = 0.086 ± 0.044 (range, 0.007–0.211), gains GF = 0.088 ± 0.045 (range, 0.000–0.215), and the cumulated index GDF = 0.174 ± 0.085 (range, 0.015–0.388). These results imply that, for the average tumor, 17% of the represented genome displayed alterations (losses or gains) at chromosomal or subchromosomal level. A strong correlation between GF and LF ($P < 0.0001$) was observed, indicating that allelic gains and losses occur concomitantly in most of the tumors. Moreover, these two indexes followed the same behavior as the cumulated GDF in most statistical tests and therefore only GDF associations will be reported.

Independence of the Different Types of Genomic Damage in Colorectal Carcinomas. As a first step, we analyzed the potential relationship between both indexes of chromosomal instability. The genomic damage detected by AP-PCR appeared to be independent of aneuploidy (both DI and AI). GDF tended to be higher in aneuploid
Genomic Damage Is Associated with Clinicopathological and Molecular Features. The most significant associations of GDF and AI with different clinicopathological and molecular parameters are summarized in Table 1. Tumors located on the left colon showed increased levels of both GDF and AI. In aneuploid tumors, advanced stages of tumors tended to display high GDF values, as expected. The prognostic factors for diploid and aneuploid tumors might be different.

Table 1 Genomic damage associations with clinicopathological and molecular variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>All tumors</th>
<th>Aneuploid tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>33</td>
<td>21</td>
</tr>
<tr>
<td>Left</td>
<td>80</td>
<td>55</td>
</tr>
<tr>
<td>Dukes' stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, B</td>
<td>58</td>
<td>39</td>
</tr>
<tr>
<td>C</td>
<td>38</td>
<td>27</td>
</tr>
<tr>
<td>D</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>p53 mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>57</td>
<td>32</td>
</tr>
<tr>
<td>Yes</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>K-ras mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>70</td>
<td>44</td>
</tr>
<tr>
<td>Yes</td>
<td>42</td>
<td>31</td>
</tr>
<tr>
<td>MSI</td>
<td>100</td>
<td>68</td>
</tr>
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</tr>
</tbody>
</table>

Values correspond to mean + SD.

Co-occurring Genetic Alterations and Clinicopathological Parameters. For practical reasons, quantitative variables were categorized into two groups of cases, defined according to the optimal cutoff point found by receiver operating characteristic analysis ("Statistical Analysis"). Cutoff value for AI was >1.343 and for GDF was >0.001. The most striking difference was the opposite effect of GDF on the survival of diploid and aneuploid tumors (Fig. 2). Cox analysis of GDF as a quantitative trait confirmed that the increase in the levels of GDF was associated with a higher risk of death for aneuploid tumors but also to a relatively lower risk for diploid tumors that were of high risk with low GDF (Fig. 3B). As a whole, these results indicate the existence of distinct invasive properties in diploid and aneuploid tumors and suggest that the prognostic factors for diploid and aneuploid tumors might be different.

Insights in the Effect of Genomic Damage on Patient Survival in Diploid Tumors. To better understand the paradigmatic overall trend of high GDF associated to better outcome in diploid tumors, we investigated the interaction GDF/p53 mutation in regard to clinical outcome. Overall survival analyses showed opposite behaviors for diploid tumors with and without p53 mutations: high GDF was an
indicator of poor prognosis (HR = 2.04) in tumors with p53 mutations (the same as aneuploid tumors) and good prognosis (HR = 0.25) in tumors with wild-type p53, although the differences were not statistically significant. This result reinforces the idea that at least two pathways are present in diploid non-MSI tumors: (a) tumors carrying p53 mutations and located in the left colon displaying similar properties as aneuploid tumors but with a better outcome; and (b) tumors without p53 mutations and low GDF but very aggressive. The rest of variables considered in diploid tumors did not show significant associations with aggressiveness.

Table 2: Comparison of bivariate and univariate models for variables that associate with aggressiveness in aneuploid tumors

<table>
<thead>
<tr>
<th>Univariate models</th>
<th>Multivariate models (maximum likelihood test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>+ Dukes</td>
</tr>
<tr>
<td>Dukes</td>
<td>0.001</td>
</tr>
<tr>
<td>p53</td>
<td>0.015</td>
</tr>
<tr>
<td>GDF</td>
<td>0.076</td>
</tr>
<tr>
<td>AI</td>
<td>0.026</td>
</tr>
</tbody>
</table>

NS, not significant.

Insights into the Effect of Genomic Damage on Patient Survival in Aneuploid Tumors. To elucidate whether distinct pathways may exist in aneuploid tumors, we analyzed the evolution of the HR in relation to GDF and AI values for this subset of tumors. In both cases, high values of genomic damage were associated with increased risk of death (GDF, P = 0.076; AI, P = 0.026), indicating a progressive aggressiveness as tumors increase their genomic disruption (Fig. 3, C and D). The bivariate model, including both GDF and AI, was significant or near-significant in regard to univariate models (Table 2), confirming the previous findings that both measurements of genomic damage have an independent effect on survival. The presence of high levels of either type of genomic damage resulted in similar overall survival rates (Fig. 4A). It is important to notice that the coincidence of both types of genomic damage in some tumors did not make them more aggressive than tumors with only one type of genomic damage.

Finally, we analyzed the combined effect of all of the parameters that are related to the survival in aneuploid tumors. Multivariate Cox
models were elaborated with the following variables: Dukes’ stage; p53 mutations; GDF; and AI (the two last considered as quantitative variables; Table 2). Two main conclusions were extracted from this analysis: (1) Despite the strong prognostic value of Dukes’ stage ($P < 0.001$), p53 mutations and GDF add information to Dukes’ univariate model ($P = 0.017$ and $P = 0.047$, respectively), indicating that both parameters have an effect on survival that is independent of Dukes’ stage (2). The prognostic value of AI overlaps with that of the Dukes’ stage and in the same way that of GDF overlaps with that of p53 mutation. This is not surprising because there are strong associations between these variables: Dukes’ D tumors display high AI levels and p53 mutated tumors display high GDF levels (Table 1).

**Genomic Damage Is the Main Predictor of Survival in R0 Aneuploid Tumors.** To further evaluate the strength of our observations and to determine the potential clinical usefulness of genomic damage assessment, statistical analysis was performed considering only R0 tumors. Despite the reduction in the number of cases, the statistical significance of associations between the two measurements of genomic damage with survival was improved: GDF log-rank, $P = 0.017$, and AI log-rank, $P = 0.007$. Multivariate analysis revealed the independence of both types of genomic damage and Dukes’ stage (Table 3). Because AI and GDF also appeared as independent parameters (see above), we combined them in a single variable that considered the presence of either type of damage. In multivariate analysis this integrated variable (AI/GDF) outperformed Dukes’ staging as a prognostic factor (Table 3). The Kaplan-Meier curve (Fig. 4B) clearly shows the striking differences in survival associated to the presence or absence of genomic damage. The combined used of the two measurements AI and GDF improves the prognostic assessment by selecting the cases without genomic damage as those with the best prognosis.

**Classification of Colorectal Carcinomas.** On the basis of the correlations observed in this study and taking into account classifications in previous studies (13–15, 17, 20, 23), we have considered a limited number (five) of variables to classify colorectal cancers: three of them (MSI, cell DNA content, and subchromosomal damage represented by GDF) characterize the profile of genomic disruption. p53 mutation is the most recurrent genetic alteration and associates with multiple features of the tumors (see below) and, finally, the site of origin of the tumor, which may have multiple etiological, biological and clinical implications.

We have used a stepwise strategy to generate the classification (schematically represented in Fig. 5) by identifying the variable or variables in each step that best define an apparently homogeneous group. In the first step, tumors with MSI (group 1) were disclosed. An intermediate step differentiated diploid and aneuploid tumors. In diploid tumors, the presence of p53 mutations appeared as the main factor identifying two groups with significant features (groups 2 and 3). In a first approach to resolve the classification of aneuploid tumors, we performed K-means cluster analysis using the four variables that were considered more likely to identify a pathway based on the observed correlations: p53 mutations; GDF; location; and DI. Two clusters arose resulting in groups comprising 61 (group 4) and 26 cases (group 5; Table 4). An alternative and simpler classification criterion (group 4, tumors meeting at least two out four conditions; left

<table>
<thead>
<tr>
<th>Categories</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukes’ stage (Astler-Coller)</td>
<td>A–B 1.8</td>
<td>0.7–4.5</td>
<td>0.185</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI</td>
<td>&lt;1.14 3.6</td>
<td>1.2–11.0</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>&gt;1.14 2.3</td>
<td>0.9–6.1</td>
<td>0.087</td>
</tr>
<tr>
<td>Dukes’ stage (Astler-Coller)</td>
<td>A–B 0.204</td>
<td>0.12–0.357</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDF</td>
<td>&lt;0.204 3.2</td>
<td>1.2–8.5</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>&gt;0.204 1.6</td>
<td>0.6–4.1</td>
<td>0.357</td>
</tr>
<tr>
<td>Dukes’ stage (Astler-Coller)</td>
<td>A–B Low 11.7</td>
<td>1.5–89.0</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDF</td>
<td>High</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Pathways of Tumor Progression in Colorectal Cancer**

<table>
<thead>
<tr>
<th>Pathways</th>
<th>Genetic alterations</th>
<th>Groups main features</th>
<th>Group classification tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI</td>
<td>Mutations in genes with mononucleotide tracts</td>
<td>Right colon, good prognosis</td>
<td>1. wild type</td>
</tr>
<tr>
<td>Stable genome</td>
<td>?</td>
<td>Right and left colon, poor prognosis</td>
<td>2. p53 mutated</td>
</tr>
<tr>
<td>Chromosome instability</td>
<td>Diploid, msp53, structural chromosomal alterations; Endoreduplication / Polyploidy</td>
<td>Left colon, good prognosis</td>
<td>3. diploid</td>
</tr>
<tr>
<td></td>
<td>High Aneuploid: msp53, structural and numerical chromosomal alterations</td>
<td>Left colon, poor prognosis</td>
<td>4. ≥2 (+)</td>
</tr>
<tr>
<td></td>
<td>Numerical chromosomal alterations</td>
<td>Right and left colon, good prognosis</td>
<td>5. &lt;2 (+)</td>
</tr>
</tbody>
</table>

Fig. 5. Scheme of the hypothesized genetic pathways of tumor progression in colorectal cancer based on a model integrating different forms of genomic instability. Each pathway is depicted according to the approximate percentage of cases displaying a given type of genetic damage. The main features of each group and the proposed decision tree used to assign each case to the groups are shown.
location, mutated p53 gene, GDF >0.204, DI >1.5; group 5, the rest) allocated all cases in an identical manner to K-means clustering. This approach results in a simplified decision tree (Fig. 5) suitable for practical applications. A summary of the main clinicopathological and genetic features of each group and the Kaplan Meier overall survival curves for each pathway are shown in Table 4 and Fig. 4C, respectively.

DISCUSSION

Genomic Instability and Progression. Colorectal cancer and other malignancies are characterized by multiple chromosomal alterations (30). The extent of chromosomal abnormalities in cancer cells has been largely documented and a correlation between increased chromosomal disruption and malignant behavior has been also reported previously (26, 27, 31–36). By analogy to MSI, Lengauer et al. (6) proposed a chromosomal instability pathway based on the heterogeneity in chromosome number observed in several human colon cancer cell lines. These authors suggested that most colorectal tumors might belong to this pathway according to the prevalence of aneuploidy, which would be the outcome of this instability. This definition of chromosomal instability was limited to numerical abnormalities, and no reference to karyotypic structural abnormalities, which are also typical in the vast majority of cancers, was made (37). Recent studies addressing the characterization of structural chromosomal instability in colorectal cancers (38–40) and cell lines (41, 42) suggest that this type of instability may play a relevant role in early and advanced stages of the progression of the disease.

If chromosomal instability drives tumor progression, the type and degree of instability are likely to leave identity marks (karyotype) in the genome of the neoplastic cell. As a whole, this will result in restricted profiles of chromosomal disruption that would be finally shaped by selection of clones most suited to survival and proliferation. The appropriate classification of the tumors is a prerequisite to identifying the components of malignancy in each pathway. The use of appropriate analytical methods to detect and/or to quantify chromosomal aberrations of a heterogeneous nature and amenable to the study of clinical specimens is therefore instrumental to the characterization and identification of distinct pathways of tumor progression.

Nature and Detection of Chromosomal Alterations. In our setting, ploidy analysis by flow cytometry and detection of allelic imbalances by AP-PCR offer simple and comprehensive approaches to the global analysis of genomic damage resulting in the generation of two independent estimates of chromosomal instability (AI and GDF). Although the aneuploidy measured by flow cytometry mostly reveals gross numerical changes (43), the GDF calculated from AP-PCR data embraces both chromosome copy number variations and simple and complex unbalanced rearrangements (44–46). In fact, serial analysis of mapped AP-PCR tags and comparison to microarray data and comparative genomic hybridization has revealed that most of the alterations detected by AP-PCR consist of allelic imbalances affecting relatively small chromosomal regions rather than whole chromosomes (45–48). Although these two estimates of genomic damage cannot be completely dissected to accurately reflect every type of chromosomal alteration separately and both types of damage coexist in some tumors, they are independent from each other as demonstrated by three facts: (a) high values of GDF do not associate with high values of AI; (b) GDF and AI show different associations with clinicopathological and molecular variables; and (c) although both types of genomic damage associate with bad prognosis, an additive effect is not observed.

Several mechanisms may underlie the chromosomal alterations detected. Chromosome number alterations have been attributed to dysfunction of the mitotic chromosome segregation apparatus (49) and endoreduplication followed by chromosome losses and stabilization at near-triploid karyotypes (14). On the other hand, structural chromosome instability is most likely to be the consequence of double strand breaks in the DNA because most of the structural aberrations involve breakage and rejoicing of DNA segments (50). Additionally somatic mitotic recombination may also contribute to generate allelic imbalances affecting limited chromosomal regions (51). The molecular basis of this cumulated chromosomal damage is unknown, but the association of increased GDF with mutations in the p53 gene suggests its implication. Although the inactivation of p53 does not seem to be the primary cause of chromosomal instability (52), it has been shown that genomic disruption coincides with the loss of p53 at the transition from preinvasive disease to invasive carcinomas (53–55). Furthermore, an association between the number of chromosome alterations (as assessed by comparative genomic hybridization) and p53 mutations in colorectal tumors has been found (56, 57). p53 plays multiple functions in the response to DNA damage (reviewed in Ref. 58) and of special interest is its role in the repair of DNA double strand break.

In vitro studies have reported that colon cancer cells carrying a mutated p53 gene acquire resistance to N-(phosphonacetyl)-L-aspartic acid through chromosome rearrangements resulting in an increased number of copies of the CAD gene, whereas in a wild-type p53 context, the parental cells undergo gains of normal chromosome 2 (59). Within this framework, our results strengthen a pivotal role of p53 inactivation in structural chromosomal instability.
Classification of Colorectal Carcinomas. Our results taken together with previous investigations advocate a classification of colorectal tumors according to their pattern of genomic disruption. These groups are likely to follow different pathways of tumor progression, which would be characterized by either different types of genomic instability or the accumulation of certain types of genetic alterations even in the absence of instability. Moreover, specific prognostic factors are revealed for each group. The quantitative nature of some measurements, the lack of complete confidence in the detection of some mutations (i.e., we have only screened for mutations in exons 4–9 of the p53 gene), and the expected heterogeneity in the genotypic and phenotypic traits of tumors belonging to the same group or pathway (which results in overlapping) precludes a perfect discrimination of the classification. To simplify the scenario, some assumptions have been made, and the classification criteria have been restricted to include a limited number of variables, which are those most likely to play a major role in the definition of our groups and, by extension, of the progression pathways. The main features of each group are discussed below.

Tumors with MSI (Group 1). It has been repeatedly reported that tumors with MSI constitute a group with well-defined characteristics (reviewed in Ref. 60); they tend to appear in younger patients, are mostly located in the right colon, and show good prognosis. MSI tumors usually bear low levels of chromosomal damage, although some of them may be aneuploid. Because of the low prevalence of MSI in the series considered here, it is not possible to discriminate prognostic factors based on our data, but other studies have already identified specific indicators of aggressiveness in these tumors (61, 62). It is also of note that tumors of this pathway may be more sensitive to alternative therapies (63, 64) and chemoprevention (65), which reinforces the interest in their appropriate identification.

Diploid Tumors without p53 Mutations (Group 2). Nineteen percent of the tumors belong to this group, which includes cancers located in the left and the right colon and showing no symptoms of microsatellite or chromosome instability. Surprisingly, these tumors show poor prognosis because they are highly aggressive once they form lymph node metastases. Other investigations have also noted that a subset of colorectal cancers are able to efficiently progress with limited or absent genomic disruption (24, 57, 66–68), supporting the need to set this group apart. Moreover, studies in a mouse model of inherited intestinal cancer [Apc(Min)−/−, Min−/++] demonstrated that adenomas progress with no need for extensive chromosomal alterations and without MSI, suggesting that genomic instability is not a prerequisite in tumor progression (11). At this time, it cannot be excluded that these tumors harbor other types of instability, for instance, in the form of point mutations or epigenetic deregulation. According to a recent study (69), there is no clear evidence for a mutator phenotype at the nucleotide level in sporadic colorectal cancer, and preliminary studies on the DNA methylation profiles of these tumors (unpublished data) have failed to identify differential epigenetic patterns in this group with regard to the rest.

Diploid Tumors with p53 Mutations (Group 3). Nine percent of the tumors are diploid, show stable microsatellites, and contain p53 mutations. The levels of chromosomal damage measured by AP-PCR are as high as in aneuploid tumors (Table 4), but its normal cell’s DNA content indicates that they have not undergone major numerical chromosomal changes. Thus, most of the observed genomic damage is likely to be of a structural nature (chromosomal rearrangements with subchromosomal losses and gains). Interestingly, tumors in group 3 apparently fit in the near diploid stage of the monosomic type defined by Dutrillaux in classical cytogenetic studies of colorectal cancers (70). Monosomic type tumors are characterized by loss of chromosomes 17p and 18, structural rearrangements, left side location, and a tendency to endoreduplication in advanced stages (14, 70). If these tumors progress without aneuploidization (remaining in group 3), the outcome is favorable, but eventually, endoreduplicated cells take over, and the tumors develop into the high aneuploid type (group 4).

High Aneuploid Tumors (Group 4). The clues suggesting the heterogeneous nature of aneuploid tumors arise mainly from two observations: the independence of the two measures of chromosomal damage—GDF and aneuploidy (either in the form of AI or DI); and the dual correlates of these two variables, specifically p53 mutations, associate with high GDF and left location associates with high DI. After the rationale of equivalences between our groups and Dutrillaux’s classification (14), high aneuploid tumors (group 4) are likely to represent the aneuploid stage of the monosomic type. The tendency of pseudodiploid monosomic tumor cells (similar to those of group 3) to undergo endoreduplication leads to pseudotetraploidy and, thereafter, a strong bias for chromosome loss results in the formation of pseudotetraploid cells (the monosomic polyplody type). As expected and in concordance with the features exhibited by tumors of group 4, monosomic polyplody tumors have a high frequency of p53 mutations, show structural chromosomal instability, are highly aneuploid, and are preferentially located in the left side. These tumors tend to have bad outcome and the main prognostic factors are the indexes of genomic damage.

Low Aneuploid Tumors (Group 5). The remaining aneuploid tumors (20% of all tumors) are located in both the right and left colon, show few p53 mutations, low chromosomal damage, and a better outcome when compared with group 4 (Table 4). Again, a correlation with the cytogenetic classification can be made: tumors in group 5 exhibited similar features to the tumors of the trisomic type identified by Dutrillaux et al. (14) and Muleris et al. (70). According to these authors, the trisomic type represents 20–25% of all tumors and is characterized by the presence of trisomies and the absence of polyploidies and structural rearrangements.

In summary, our results support the concept of colorectal cancer as a heterogeneous disease. Different mechanisms are likely to underlie colorectal tumor progression and result in alternative pathways. Tumors of each pathway would display characteristic genetic profiles and differentiated biological behavior. Therefore, the observed molecular and clinicopathological correlates are better understood after classification of cancers in subsets representing the postulated pathways of progression. Although different factors are likely to confuse the classification (i.e., shared features, overlapping between pathways, convergent stepwise progression, and so forth), specific signs or marks should also be preserved allowing the identification of the most probable pathway of progression. We have used estimates of global genomic disruption in its different forms (chromosomal, subchromosomal, and at the sequence level) as a starting point to define groups based upon the premise that the pattern of genetic alterations is likely to bear the marks of the agent driving progression (i.e., genomic instability) and can also be linked to many biological features of the tumor. It is obvious that this classification must be refined with additional genetic analyses (i.e., comparative genomic hybridization and profiles of gene expression) before it may have an actual application in clinical prognostic assessment and patient management. However, it is also clear that the subtypes of colorectal cancers proposed here are likely to constitute the core of actual pathways of tumor progression. Future studies on prognostic assessment and response to treatment should take into account the particularities of each group.

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GENETIC PATHWAYS IN COLORECTAL CANCER

REFERENCES


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