Exposure to the Tobacco Smoke Constituent 4-Aminobiphenyl Induces Chromosomal Instability in Human Cancer Cells

Federica Saletta,1 Giuseppe Matullo,2,3 Maurizio Manuguerra,1 Sabrina Arena,3 Alberto Bardelli,1,4 and Paolo Vineis1,5

Abstract

The relationships between environmental factors and the genetic abnormalities that drive carcinogenesis are supported by experimental and epidemiologic evidence but their molecular basis has not been fully elucidated. At the genomic level, most human cancers display either chromosomal (CIN) or microsatellite (MIN) instability. The molecular mechanisms through which normal cells acquire these forms of instability are largely unknown. The arylamine 4-aminobiphenyl (4-ABP) is a tobacco smoke constituent, an environmental contaminant, and a well-established carcinogen in humans. Among others, bladder, lung, colon, and breast cancers have been associated with 4-ABP. We have investigated the effects of 4-ABP and \(^{N}\)-nitro-\(^{N}\)-nitrosoguanidine (MNNG) on genetically stable colorectal (HCT116) and bladder (RT112) cancer cells. Cells were treated with carcinogens to generate resistant clones that were then subjected to genetic analysis to assess whether they displayed either CIN or MIN. We found that 50% to 60% of cells treated with 4-ABP developed CIN but none developed MIN as confirmed by their ability to gain and lose chromosomes. In contrast, all MNNG-treated clones (12/12) developed MIN but none developed CIN as shown by the microsatellite assay. The mismatch repair protein expression analysis suggests that the acquired mechanism of MIN resistance in the HCT116 MNNG-treated cells is associated with the reduction or the complete loss of MLH1 expression. By providing a mechanistic link between exposure to a tobacco constituent and the development of CIN, our results contribute to a better understanding of the origins of genetic instability, one of the remaining unsolved problems in cancer research. [Cancer Res 2007;67(15):7088–94]

Introduction

Carcinogenesis has been interpreted for a long time as the effect of point mutations. However, Cairns (1) pointed out long ago that it is unlikely that mutagenesis alone explains carcinogenesis. He suggested that the driving forces in the process are more likely to be gross chromosomal abnormalities and the selection of cells that carry them. Such interpretation has received support from recent observational, experimental, and theoretical developments: (a) the observation of an association between chromosome abnormalities and cancer in prospective studies in humans (2); (b) the acknowledgment of the importance of genetic instability in hereditary and sporadic cancers, including chromosome instability (CIN) and microsatellite instability (MIN; refs. 3, 4); and (c) a “Darwinian” interpretation of the carcinogenic process, in which a key role is played by the selective advantage of cells carrying genetic abnormalities (5–7).

The present study had two aims: (a) to investigate the mechanisms of acquired resistance to treatment with high doses of potent carcinogens, and (b) to investigate the types of genetic instability (CIN or MIN) acquired by cells which became resistant to different carcinogens.

Epidemiologic studies indicate that a large fraction of human cancers are associated with exposure to specific environmental carcinogens. For example, the occurrence of human bladder cancer is largely explained by exposure to arylamines from different sources, including tobacco-smoking and occupational exposures. The arylamine 4-aminobiphenyl (4-ABP) is a tobacco smoke constituent, an environmental contaminant, and a well-established carcinogen in humans. Among others, bladder, lung, colon, and breast cancers have been associated with 4-ABP. Previous research from our group and other groups has suggested that arylamines contained in tobacco smoke—such as 4-ABP—form adducts with proteins and DNA, can induce p53 mutations in the bladder, and eventually lead to bladder cancer (8–11). 4-ABP is an environmental contaminant generated mainly from cigarette smoke, the combustion of fossil fuels, as well as in rubber and coal (12). Upon metabolic activation, the electrophilic derivatives of 4-ABP interact with DNA and form adducts that have been found to correlate with bladder carcinogenesis in humans and animals (13). Thus far, it was believed that the molecular bases through which 4-ABP mediates its carcinogenic activity were connected to its ability to cause mutations in the human genome (14, 15).

There is evidence, however, that bladder cancer is mainly the result of gross chromosome alterations rather than point mutations. Chromosome aberrations and chromosome instability (CIN) have been shown to play a central role in the onset of most solid tumors. A smaller fraction of cancers are associated with mismatch repair defects and consequently display MIN. We previously reported that exposure to specific carcinogens can select for tumor cells with distinct forms of genetic instability (CIN or MIN; refs. 16–18). We therefore hypothesized the existence of a mechanistic link between tobacco smoke constituents—such as 4-ABP—and the occurrence of CIN in cancer cells. To test this hypothesis, we took advantage of genetically stable bladder (RT112) and colorectal (HCT116-CH3) cell lines. These cells represent two useful models to assess the relationship between carcinogens and genetic instability as they can manifest CIN or MIN, but not both (3, 19). In addition, both cell lines were completely stable in terms of maintenance of chromosome number and nucleotide sequence.
Materials and Methods

**Study design.** The study was designed according to a two-step strategy. First, genetically stable human cancer cells were treated with specific carcinogens [4-ABP or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)] to generate resistant clones. Second, we assessed whether carcinogen-resistant cells displayed either CIN or MIN, after a second treatment with the same carcinogen.

**Cell culture.** HCT116 chromosome 3 corrected cells were cultured in McCoy’s 5A medium (Sigma) supplemented with 10% (vol/vol) fetal bovine serum (Sigma), 1% penicillin-streptomycin solution (10,000 units penicillin G and 10 mg streptomycin/mL; Sigma). RT112 cells were cultured in RPMI 1640 supplemented with 10% FBS (vol/vol), 1% penicillin-streptomycin solution (10,000 units penicillin G and 10 mg streptomycin/mL).

**Carcinogen treatment.** For treatment with 4-ABP (Sigma), cells were detached, counted, and resuspended in serum-free RPMI 1640. For treatment with MNNG (Sigma), cells were detached, counted, and resuspended in serum-free RPMI 1640. Cells were treated for 4 h at 37°C, and then washed and resuspended in complete medium. 4-ABP-resistant cells were obtained by treatment with 125 μg/mL of 4-ABP for RT112 cells and 175 μg/mL of 4-ABP for HCT116 cells. After treatment, single-cell clones were obtained by a limiting dilution.

For treatment with MNNG (Sigma), cells were detached, counted, and resuspended in serum-free RPMI 1640. Cells were treated for 4 h at 37°C, and then washed and resuspended in complete medium. MNNG-resistant cells were established by treatment with 0.75 μg/mL of MNNG for RT112 cells and 1.0 μg/mL of MNNG for HCT116 cells. Twenty-four hours after treatment, cells were detached and resuspended in fresh medium. After another 24 h, single-cell clones were obtained by a limiting dilution. The relative resistance of isolated clones was evaluated by the treatment of 5 × 10^6 cells with appropriate concentrations of carcinogens. After exposure to the carcinogen, cells were detached, stained with 2% (vol/vol) trypan blue (Sigma) and re-counted. Two subsequent treatments were necessary to increase clone resistance.

**MIN assay.** All clones, as well as the parental cell lines, were screened for sequence alterations in the mononucleotide repeat BAT-26, located within intron 5 of the MSH2 gene (20). Assessment of the status of BAT-26 has been previously used to establish MIN status (21). PCR reactions were done in 12.5 μL reactions containing 2.5 μL of genomic DNA (extract with QIAmp column; Qiagen), 1.25 μL of buffer, 1.25 μL of MgCl2, 200 μmol/L of deoxynucleotide triphosphates, and 0.5 μmol/L of each primer. The primers used for amplification were 5’-TTT GAC TAC TTT TGA CTT CAG CC-3’ and 5’-AAC CAT TCA ACA TTT TTA ACC C-3’. Forward primers were fluorescently-labeled at the 5’-end. Initial denaturation was done at 95°C for 12 min, followed by 10 cycles of 15 s at 95°C, 15 s at 54°C, and 30 s at 72°C and other 30 cycles of 15 s at 89°C, 15 s at 54°C, and 30 s at 72°C with a final extension step at 72°C for 30 min. Diluted PCR products (1 μL) were mixed with 12 μL of formamide and 0.5 μL of internal standard size maker (TAMRA 500). The mix was denatured at 95°C for 5 min and cooled on ice. Samples were loaded on an ABI 377 DNA sequencer (Applied Biosystems). The data were collected automatically and analyzed using the Genescan 3.1 software (ABI). MIN was scored if microsatellite lengths in clones and matched normal cells were different.

**Fluorescence in situ hybridization.** Carnoy-fixed cells were centrifuged and fresh fixative was added to obtain the desired cell concentrations for fluorescence in situ hybridization (FISH) analysis. Single-slide multi-chromosome FISH analysis was done using the Chromoprobe Multiprobe system following the manufacturer’s protocol (Cytocell). The Chromoprobe

### Table 1. Development of carcinogen-resistant cell lines

<table>
<thead>
<tr>
<th>Clone name</th>
<th>Parental cells</th>
<th>Description</th>
<th>Mortality</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT116</td>
<td>HCT116-H3 corrected</td>
<td>Parental culture*</td>
<td>99.3</td>
<td></td>
</tr>
<tr>
<td>HCT-4ABP-5</td>
<td>HCT116-H3 corrected</td>
<td>Clone †</td>
<td>55.5</td>
<td>&lt;0.0001</td>
</tr>
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<td>HCT-4ABP-8</td>
<td>HCT116-H3 corrected</td>
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<td>57.3</td>
<td>&lt;0.0001</td>
</tr>
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<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
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<tr>
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<td>RT112</td>
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<td>&lt;0.0001</td>
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<td>Clone †</td>
<td>57.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RT-MNNG-8</td>
<td>RT112</td>
<td>Clone †</td>
<td>58.2</td>
<td>&lt;0.0001</td>
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<td>74.7</td>
<td>&lt;0.0001</td>
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</table>

NOTE: Comparison of mortality between parental cultures and clones derived from treatments with carcinogens. \( P \) values were calculated with the Fisher exact test under the null hypothesis of no difference in mortality between treated clones and the parental culture. Mortality percentages are referred to the second carcinogen treatment.

* Mortality is expressed as a cumulative risk in comparison with the original untreated line (first treatment).
† Mortality is expressed as a cumulative risk in comparison with the parental culture (second treatment).
Multprobe-I system allows the simultaneous enumeration of up to 24 chromosomes. The device was divided into 24 square areas each containing a satellite III probe (chromosomes 1, 9, and Y) or an α-satellite one (all the other chromosomes). Some probes recognized more than one chromosome, and for this reason, it is not possible to discriminate between chromosomes 1, 5, and 19, chromosomes 13 and 21, and chromosomes 14 and 22. Moreover, centromere signals for chromosomes 16, 17, 20, and Y were particularly weak. We decided to limit our CIN assay to chromosomes from numbers 2 to 12, excluding chromosome number 5 and confirming results using chromosome-specific painting probes (CytoCell).

For FISH analysis, cell suspensions were dropped on clean and dry slides and slides were immersed in 2× SSC (pH 7.0) for 2 min and then dehydrated in an ethanol series (70%–85%–100%), for 2 min each. Ten microliters of probe were placed on cell sample slides, covered with glass coverslips and end-sealed with rubber cement. Slides were denaturated onto a hotplate at 75°C for 2 min and then placed overnight in a humid, light-proof chamber at 37°C. The day after, coverslips were removed and slides washed in 0.4× SSC at 72°C for 2 min, and then in 2× SSC and 0.05% Tween 20 at room temperature for 30 s. Ten microliters of 4′,6-diamidino-2-phenylindole antifade were then applied on dry slides and covered with coverslips. Results were observed with the DM2000 fluorescence microscope (Leika) and images were collected using the FW400I software (Leika).

**Immunoblotting.** Cell lysates were prepared in EB buffer. Immunoblotting was done on Hybond membrane (Amersham). Anti-MLH1 and anti-MSH2 antibodies (PharMingen) were used according to the instructions of the manufacturer. Signals were developed by using enhanced chemiluminescence (Perkin-Elmer). Relative quantification of protein expression was done using Quantity One v.4.6 Basic software (Bio-Rad).

**Statistical analyses.** The statistical significance of the differences between the resistance of clones (second treatment) and parental cell lines was tested using a two-sided P value from a Fisher test applied to a cell-counting data (Table 1). The same test was used to evaluate the differences in MIN between resistant clones and parental cell lines. CIN was assumed if at least 40% of the investigated markers (chromosomes) revealed loss of signal.

A homoscedastic two-tailed t test was used to investigate the statistical significance of the differences between the cell line and the clone protein expression, as quantified by the immunoblotting method. The variance used in the t test has been estimated from repeated measures of the same signals, to take into account the variability derived from the Bio-Rad algorithm quantifying protein expression. All calculations were done in R version 2.4.1.

**Results**

**Development of isogenic cellular models displaying resistance to human carcinogens.** To assess whether genetic instability could be associated with exposure to carcinogens, we took advantage of two cell models. The cell line HCT116 was originally derived from a MIN colorectal cancer carrying a point mutation in the mismatch repair gene hMLH1 (22). MIN in these cells was corrected by adding an extra chromosome 3 containing the hMLH1 wild-type gene (23). RT112 is a DNA repair–proficient cell line derived from a human epithelial bladder carcinoma (24) that we found to be chromosomally stable.

The first step of this work was to establish derivatives of the two cell lines displaying resistance to the two carcinogens (4-ABP and MNNG). To achieve this, we treated the cells with a panel of carcinogen concentrations in order to obtain cytotoxicity curves for every combination of cell line carcinogen (Fig. 1).

Then, 4-ABP-resistant and MNNG-resistant cells were established by treatment with the concentration of carcinogens that normally kills >99% of cells of the two cell lines (HCT116 and RT112). After treatment, viable single-cell clones were obtained by limiting dilution.

### Table 2. Measurement of CIN

<table>
<thead>
<tr>
<th>Clone name</th>
<th>%CIN (CHR 11)</th>
<th>%CIN (CHR 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT116</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HCT-4ABP-5</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>HCT-4ABP-8</td>
<td>62</td>
<td>52</td>
</tr>
<tr>
<td>HCT-4ABP-10</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>HCT-4ABP-11</td>
<td>62</td>
<td>48</td>
</tr>
<tr>
<td>HCT116</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HCT-MNNG-5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>HCT-MNNG-8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>HCT-MNNG-10</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>HCT-MNNG-12</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>RT112</td>
<td>0</td>
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</tr>
<tr>
<td>RT-4ABP-2</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
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<td>RT-4ABP-12</td>
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<tr>
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<td>0</td>
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</tr>
<tr>
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<tr>
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<td>4</td>
</tr>
<tr>
<td>RT-MNNG-12</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

**NOTE:** Levels of CIN displayed by carcinogen-resistant clones. Percentage of subnormal chromosomal signals out of 50 interphases observed in parental and carcinogen-treated cell lines.
The relative resistance of isolated clones to the carcinogens was assessed by using the trypan blue viability assay (Table 1). We identified at least four independent cell sublines showing a significant degree of resistance as measured by the decrease (from >99–50% to 60%; \( P < 0.0001 \)) of the carcinogen-associated mortality (Table 1). Clones that survived treatment but did not acquire resistance were not analyzed further.

**Cells exposed to the tobacco smoke constituent 4-ABP display CIN.** To determine whether the carcinogens 4-ABP and MNNG had induced CIN in the treated cells, we did a FISH assay
corresponding parental counterparts (Fig. 2).

Loss of chromosomes 4 and 6 when compared with the parental HCT116 and RT112 cell lines.

A

and resistant to 4-ABP showed selective loss of chromosomes 11 and 20. For example, HCT116 clones acquired CIN. These results indicate that cells exposed to 4-ABP are capable of assessing CIN by measurement of random chromosome losses and gains. To establish the assay, we first did a preliminary karyotype analysis that confirmed trisomy of chromosome 3 and monosomy of chromosome 21 in the HCT116 cells. We also found that RT112 cell line displayed monosomy of chromosome 17, a typical aberration in bladder cancers.

Next, CIN was assessed using a FISH strategy, allowing the concomitant analysis of all 24 chromosomes (see Materials and Methods for details on the CIN assay). The results of the assay, confirmed by using chromosome-specific painting probes, indicate that 4-ABP–resistant clones (obtained from both cell lines) display a striking and reproducible CIN phenotype consisting of the ability of losing and gaining chromosomes (Table 2). On the contrary, the parental cell lines and those resistant to MNNG were chromosomally stable. These results indicate that cells exposed to 4-ABP acquire CIN.

The FISH analysis also suggested specific differences between the clones treated with 4-ABP. For example, HCT116 clones resistant to 4-ABP showed selective loss of chromosomes 11 and 20 (Fig. 2A and B; Table 2), whereas RT112 clones showed selective loss of chromosomes 4 and 6 when compared with the corresponding parental counterparts (Fig. 2C and D; Table 2).

Cells exposed to the carcinogen MNNG display MIN. It has been described that MIN can be assessed by analyzing tumor DNA with specific markers such as the BAT-26 microsatellite located in intron 5 of the MSH2 gene (see ref. 26 for more details). To determine whether the carcinogen-resistant clones showed MIN, we extracted their genomic DNA and amplified target sequences with FAM-labeled BAT-26 primers. Although the BAT-26 sequence was stable in the parental cell lines and in all 4-ABP–resistant clones, all MNNG-resistant clones (derived from both HCT116 and RT112) displayed a striking degree of MIN (P < 0.0001: Fig. 3).

Because MIN has previously been linked to mismatch repair defects, we have used Western blotting to analyze the level and pattern of expression of MLH1 and MSH2 in clones resistant to the carcinogens. The results suggest that the acquired mechanism of MIN resistance in the MNNG-treated colorectal cells is associated with the reduction or the complete loss of MLH1 expression. In particular, clone HCT-MNNG-5 has a protein expression level that is not statistically different from the parental cell line (P = 0.758), whereas clones HCT-MNNG-8 and HCT-MNNG-10 show a reduction of the expression levels (P = 0.002 and P = 0.045, respectively). In clone HCT-MNNG-12, the signal is absent (P < 0.001).

Interestingly, the mechanism seems to be cell line–specific and carcinogen-specific, whereas the same does not occur in RT112 cells line and in 4-ABP–resistant clones (data not shown). The results are shown in Fig. 4.

Carcinogen-specific induction of CIN and MIN. Most, if not all, human cancers display either CIN or MIN. Interestingly, the two forms of instability seem to be mutually exclusive. For example, ~85% of colorectal cancers are CIN with the remaining 15% displaying MIN. This suggests that one of the forms of genetic instability is sufficient to drive tumorigenesis and that CIN and MIN cancers may be epidemiologically associated with exposure to specific environmental carcinogens. We took advantage of the cells treated with the different carcinogens to assess whether the “genetic instability phenotype” was carcinogen-specific. To this end, we verified whether cells that had become CIN upon exposure to 4-ABP may also display MIN and vice versa. We found that the two forms of genetic instability were present in a mutually exclusive pattern in our isogenic cell models. Specifically, cells exposed to 4-ABP developed CIN but not MIN, as shown by analyzing their BAT-26 sequence (Figs. 2 and 3; Table 2). On the contrary, cells exposed to MNNG developed MIN but not CIN as measured using the chromosomal gain and loss FISH assay previously described (Figs. 2 and 3; Table 2).

Discussion

CIN and MIN have been described as two alternative pathways to cancer. Both are mainly referred to as intrinsic properties of the cells that display them, as traits that persist throughout the lifetime of the cells, and as a property that the cells can transmit to their progeny. CIN is generally defined as the ability of a cell to gain and lose chromosomes and is a feature of many (both sporadic and hereditary) types of cancer (3, 27). Conversely, MIN is mainly
associated with hereditary nonpolyposis colorectal cancer and sporadic gastrointestinal cancers carrying a defect in the DNA mismatch repair machinery (MIN cancers). The assessment of MIN status aids in establishing a clinical prognosis and may be predictive of tumor response to chemotherapy.

The present study aimed at investigating the ability of different carcinogens to cause CIN or MIN, respectively, in cultured cell lines. The arylamine 4-ABP is a tobacco smoke constituent, an environmental contaminant, and is a well-established carcinogen in humans. Bladder, lung, colon, and breast cancers have been associated with 4-ABP (28–31). The molecular basis through which 4-ABP mediates its carcinogenic activity is poorly understood and are thought to be connected to its ability to cause mutations in the human genome. We hypothesized that in addition, or independently from its activity as a direct DNA mutagen, 4-ABP could affect tumor occurrence by inducing genetic instability. Our hypothesis was based on the evidence that bulky adduct-forming agents, such as 4-ABP, can induce chromosome breaks through nucleotide excision-repair processes (32), leading to the suggestion that cells with defects in DNA repair or mitotic checkpoints might be selected after exposure to such agents (15, 33).

Our results show that upon exposure to 4-ABP, human cells become genetically unstable. Specifically, they display CIN as confirmed by their ability to gain and lose chromosomes at a high frequency. CIN represents the predominant form of genetic instability in human solid tumors (including bladder and colon), whereas MIN occurs in only 10% to 15% of sporadic colorectal cancers and is rare in other tumors (30). Interestingly, the net result of CIN is the deregulation of chromosome number (aneuploidy) and an enhanced rate of loss of heterozygosity, which is an important mechanism of inactivation of tumor suppressor genes. Cytogenetic studies of bladder, lung, and colon tumors have shown that karyotype complexity, cell ploidy, and the number of structural changes found were closely associated with tumor grade and stage (34–39).

Previous studies on bladder cancer have detected complex patterns of losses and gains of chromosomes (40–44). Interestingly, the selective losses of chromosomes 4 and 6 that we observed in the carcinogen-resistant cell lines indeed corresponded with some of the most common losses typically observed in bladder cancers. Importantly, in the parental RT112 line, these alterations were not present. In the colorectal cancer cell line, we detected possible selective losses of chromosomes 11 and 20. Previous studies on colorectal cancers (45–49) showed complex cytogenetic profiles in which the gain of chromosomes 20, 7, and 13, and the loss of chromosomes 4 and 18, were commonly observed. Interestingly, the selective losses of chromosomes 11 and 20 have also been previously detected.

Altogether, our results indicate that the chromosomal alterations associated with the acquisition of resistance to carcinogens might be tumor-specific and cell-specific. Further studies are clearly needed to confirm a possible mechanistic role for the specific chromosomal alterations we detected and the development of carcinogen resistance and genomic instability. The mismatch repair protein expression analysis suggests that the acquired mechanism of MIN resistance in the HCT116 MNNG-treated cells is associated with the reduction or the complete loss of MLH1 expression. The mechanism seems to be carcinogen-specific as the same does not occur in 4-ABP–resistant clones.

The dose of carcinogen was high enough to kill >99% of the cells of the two cell lines used. These exposure circumstances are obviously far from most human experiences of exposure to carcinogens. Cumulative exposure to tobacco carcinogens can easily approach or even overtake a total dose that would kill 99% of cells in the tissue, but chronic cumulative exposure is not comparable to acute exposure. Cells chronically exposed to the same cumulative dose have time to repair and do not undergo massive death. Further investigations are planned with chronic exposures of cells to lower doses. However, a more direct comparison with the human exposure situation is possible if we consider acute exposures that might massively affect certain tissues, such as the exposure of bladder cells to occupational carcinogens in the past (including aromatic amines), or the exposure of liver cells to aflatoxin. In fact, a few experimental studies have considered the effects of acute, high-dose exposures in animals, and concluded that this could be a plausible mechanism of carcinogenesis in addition to the more conventional one based on long-term exposure to lower doses (50). In these cases, our model of acquired genomic instability followed by clone selection may be more directly relevant.

We therefore propose that CIN in tumors such as those affecting the bladder and the colon might be the result of exposure to the tobacco smoke constituent and environmental pollutant 4-ABP or chemicals with a similar mechanism of action. Furthermore, we suggest that different environmental carcinogens can induce specific forms of genetic instability (14, 15). These data offer potential clues to one of the remaining unsolved problems in cancer research, the relationship between environmental factors and the genetic abnormalities that drive tumorigenesis. By providing a mechanistic link between exposure to a tobacco constituent and the development of CIN, our results contribute to a better understanding of the origins of genomic instability, a key but thus far, rather mysterious concept in carcinogenesis.

Acknowledgments

Received 12/1/2006; revised 3/30/2007; accepted 4/19/2007.

Grant support: Compagnia di San Paolo (Torino), the Italian Association for Cancer Research, the Italian Technology and Research Ministry, the Regione Piemonte and partly by Environmental Cancer Risk, Nutrition, and Individual Susceptibility, a network of excellence operating within the European Union 6th Framework Program, Priority 5 “Food Quality and Safety” (contract no. 513943).

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The authors thank Dr. Giorgio Ponzo for his excellent technical cytogenetical assistance in classical karyotyping and Dr. Ludovica Verdun di Contogno for her helpful suggestions on FISH analysis.

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