

# *p53*-Mutant Clones and Field Effects in Barrett's Esophagus<sup>1</sup>

Laura J. Prevo, Carissa A. Sanchez, Patricia C. Galipeau, and Brian J. Reid<sup>2</sup>

Programs in Cancer Biology and Gastrointestinal Oncology, Divisions of Human Biology and Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 [L. J. P., C. A. S., P. C. G., B. J. R.], and Departments of Genetics and Medicine, Gastroenterology Division, University of Washington Medical Center, Seattle, Washington 98195 [B. J. R.]

## Abstract

Previous studies have demonstrated multifocal neoplasia in Barrett's esophagus. We evaluated 213 mapped, flow-purified, endoscopic biopsies to determine the distribution of *p53*-mutant clones in the Barrett's segments of 58 patients who had high-grade dysplasia without cancer. Twenty-nine patients (50%) had *p53* mutations in their Barrett's segments, including 3 patients with multiple distinct *p53* mutations. *p53*-mutant clones, including diploid cell populations, underwent expansion from 1 to 9 cm in the Barrett's segment. In 12 of 29 patients (41%) with a *p53* mutation, the same mutation was found at every evaluated level of the metaplastic epithelium. This extensive *p53*-mutant clonal expansion suggests a somatic genetic basis for previous observations of field effects in Barrett's esophagus.

## Introduction

The incidence of esophageal adenocarcinoma has been increasing rapidly in the United States and several regions of Western Europe since the 1970s (1). Barrett's esophagus, the only known precursor to esophageal adenocarcinoma, is a premalignant metaplasia characterized by replacement of the normal stratified squamous epithelium with a specialized columnar epithelium (2). Several previous studies have demonstrated multifocal high-grade dysplasia and cancer in Barrett's esophagus, suggesting a field effect for carcinogenesis in this condition (2, 3).

The *p53* tumor suppressor gene, located on chromosome 17p13, is inactivated in Barrett's esophagus by mutation of one allele and loss of the remaining 17p allele (17p LOH<sup>3</sup>; Refs. 4–7). Abnormalities involving *p53* (mutation; 17p LOH) are common in esophageal adenocarcinoma and have been detected in premalignant tissue surrounding cancer in esophagectomy specimens (4–6, 8). Although *p53* has been studied extensively in patients who have already developed cancer, relatively little is known concerning the biological behavior of somatic *p53*-mutant clones in patients who have Barrett's esophagus without cancer *in vivo*. In two patients, we recently demonstrated expansion of *p53*-mutant clones with 8- and 11-cm Barrett's esophageal segments 6 and 3 years before the development of cancer (9). This raises the possibility that *p53*-mutant clonal expansion is a frequent, early event in neoplastic progression in Barrett's esophagus. However, there have been no systematic reports of the distribution of *p53* mutations in the Barrett's segment of patients without cancer. Therefore, we investigated *p53*-mutant clonal expansion in 213 mapped, flow-sorted, endoscopic biopsies obtained from 58 patients who had high-grade dysplasia without cancer in Barrett's esophagus. Our results indicate that *p53*-mutant clones undergo expansion involv-

ing variable regions of esophageal mucosa, thereby creating an abnormal clonal field that can evolve increased 4N fractions and aneuploid cell populations in the absence of cancer.

## Materials and Methods

**Patient Samples.** The Seattle Barrett's Esophagus Study was approved by the Human Subjects Review Boards at the University of Washington and the Fred Hutchinson Cancer Research Center. Patients were counseled concerning the risks and benefits of endoscopic surveillance and alternatives, including esophagectomy for high-grade dysplasia. Tissue was evaluated from 61 consecutive patients who had a maximum histological diagnosis of high-grade dysplasia in at least one biopsy at an endoscopy in the Seattle Barrett's Esophagus Study between January 1995 and September 1998 and who did not have cancer. A sufficient sample was available for *p53* sequence analysis in 58 of the 61 patients. Mapped endoscopic biopsies were obtained for histology and flow cytometry at 1- to 2-cm intervals of the Barrett's segment (10). Exons 5 through 9 of *p53* were typically sequenced in at least one flow-purified sample from every 2-cm level of the Barrett's segment. Two hundred thirteen flow-purified samples from 58 patients were evaluated.

**Flow Cytometric Sorting.** Neoplastic cell populations were purified from biopsies obtained at 2-cm intervals of the Barrett's segment by sorting diploid G<sub>1</sub>, 4N, or aneuploid cell populations using Ki67/DNA content multiparameter flow cytometry (11). 4N fractions >6% were classified as abnormal (10).

**DNA Sequencing.** Using genomic DNA extracted from 1000–3000 sorted cells according to our protocols, exons 5 through 9 of the *p53* gene were amplified by PCR as a single product according to published methods (11, 12). PCR products were purified using Microcon-100 filters (Amicon BioSeparations, Beverly, MA) according to the manufacturer's instructions. Using the purified PCR products, *p53* exons 5 through 9 were amplified as three separate PCR products (exons 5–6, 7, and 8–9) and sequenced as described previously (4). All mutations were confirmed by at least two independent PCR reactions. Wild-type sequences were confirmed for all patients using constitutive samples.

**Statistical Analysis.** Pearson  $\chi^2$  test for comparison of proportions was used to compare the prevalence of *p53* mutations in diploid and nondiploid cell populations.

## Results

Twenty-nine of 58 patients (50%) with high-grade dysplasia and no cancer in Barrett's esophagus had one or more somatic mutations in exons 5 through 9 of the *p53* gene. Three patients had multiple *p53* mutations, including one patient, case 333, with four different *p53*-mutant clones, resulting in 34 separate *p53* mutations in the 29 patients (Table 1). The types of mutations found in premalignant tissue were similar to those reported previously in esophageal adenocarcinomas. We found 74% transitions (25 of 34, including four nonsense), 15% deletions (5 of 34), 3% insertions (1 of 34), 3% complex mutations (1 of 34, two consecutive transitions, including one nonsense), and 6% transversions (2 of 34) compared with 67–94, 0–11, 0–22, 0, and 0–18%, respectively, in esophageal adenocarcinoma (4–6, 8, 13, 14). More than 32% of the mutations (11 of 34) were nonsense, insertions, or deletions.

Clones containing *p53* mutations underwent variable expansion in the Barrett's segment (Fig. 1). In one patient, the same mutation was

Received 6/24/99; accepted 8/17/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> Supported by NIH Grant RO1 CA61202.

<sup>2</sup> To whom requests for reprints should be addressed, at Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, P. O. Box 19024, Seattle, Washington 98109-1024. Phone: (206) 667-2875; Fax: (206) 667-6132.

<sup>3</sup> The abbreviation used is: LOH, loss of heterozygosity.

found over 9 cm of a Barrett's segment, whereas the mutation was localized to a single level in other cases. There was a striking tendency for p53-mutant clones to expand throughout the Barrett's segment. The same p53 mutation was found at every evaluated level of the metaplastic epithelium in 12 of the 29 patients (41%; Figs. 1 and 2, B, G, H, I, and J).

In 10 of the 29 cases (35%) who had a p53 mutation, the mutations were detected in premalignant diploid cell populations (Table 1, 2N Ploidy). Some of these p53-mutant diploid cell populations were localized to a single level of the Barrett's segment (Fig. 2C), whereas others underwent extensive clonal expansion in the absence of increased 4N fractions, aneuploidy, or cancer (Fig. 2B). In other cases, a diploid p53-mutant clone appeared to spread over variable regions of esophageal mucosa before giving rise to an aneuploid cell population (Fig. 2J). In yet other cases, the p53-mutant clone was already aneuploid by the time it was detected (Fig. 2, D, H, and I). One patient had four different aneuploid cell populations, each with a different p53 mutation, within a 3-cm region of Barrett's esophagus (Fig. 2E). We also detected p53-mutant increased 4N populations adjacent to aneuploid (Fig. 2A) and diploid (Fig. 2, F and G) populations sharing the same mutation.

p53 mutations were more prevalent in patients with aneuploidy or increased 4N fractions but occurred in the absence of either flow cytometric abnormality. Aneuploid cell populations were present in 25 of the 58 patients (43%). Twenty-one of 25 patients (84%) with aneuploidy had p53 mutations compared with only 8 of 33 patients (24%) without aneuploidy (P << 0.001). Eight of the 33 patients without aneuploidy had increased 4N fractions, and four of these eight (50%) had p53 mutations. Thus, the prevalence of p53 mutations in

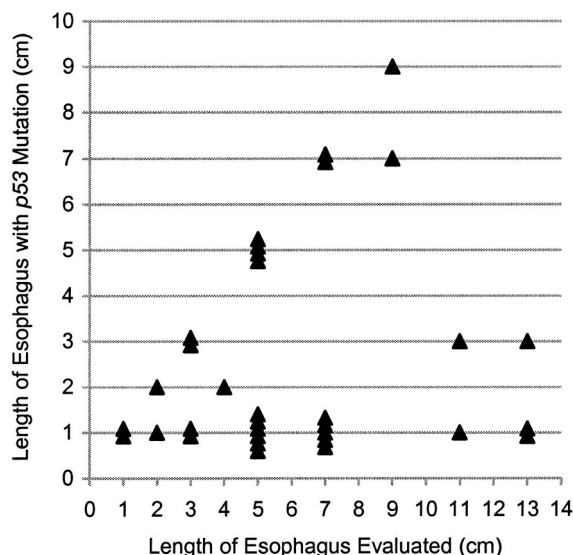


Fig. 1. Clonal expansion of p53-mutant populations in Barrett's esophagus. Exons 5 through 9 of the p53 gene were sequenced in biopsies taken at 2-cm intervals of the Barrett's segment using flow cytometrically purified samples. Each point represents a unique p53-mutant population; therefore, patients with multiple mutations will have more than one point (except patient 336, in whom two p53 mutations are present in the same clone).

patients with either aneuploidy or increased 4N was 25 of 33 (76%) compared with 4 of 25 (16%) in patients with only diploid cell populations (P << 0.001).

Discussion

Previous studies of p53 mutations in Barrett's esophagus have focused on patients who had already progressed to cancer (4–6, 8, 13, 14). Our results in 58 well-characterized patients who had high-grade dysplasia without cancer in Barrett's esophagus extend our understanding of the biological behavior of p53-mutant clones in a human premalignant epithelium. Our results demonstrate extensive expansion of p53-mutant clones to occupy fields involving up to 9 cm of esophageal mucosa, even in the absence of flow cytometric abnormalities and cancer. The factors that determine clonal expansion of p53-mutant cells versus clonal evolution of normal cells to abnormal 4N fractions and aneuploidy are presently unknown. These factors may be stochastic or the result of environmental carcinogens that cause DNA damage and progression to aneuploidy when the cell cycle checkpoint functions of p53 are inactivated (15). However, it is also possible that p53 mutations are easier to detect than cytometric abnormalities, especially increased 4N fractions, resulting in more false-negative 4Ns than false-negative p53 mutations. In 4 of the 58 cases evaluated, we observed expansion of aneuploid populations that did not have p53 mutations in exons 5 through 9, suggesting that p53 mutation may not be necessary for either aneuploidy or clonal expansion in Barrett's esophagus. It also remains possible in these cases that p53 mutations are present in exons not screened, p53 is disrupted by an alternate mechanism, or another gene in the same pathway is inactivated.

Cancers of the head and neck, lung, and esophagus appear to be associated with a field effect in which development of malignancy at one site is associated with an increased risk of a second cancer at another site (16–19). Although this is less frequently considered for Barrett's esophagus, we and others have observed multiple neoplasms in the same patient's Barrett's segment (2, 3).<sup>4</sup> The nature of these

Table 1 p53 mutations in patients with high-grade dysplasia in Barrett's esophagus

Case	Ploidy <sup>a</sup>	Nucleotide change	Amino acid change
129	2N	388C→T	Leu130Phe
181	3.4N, 3.7N	644–653delGTGTGGTGGT	Ser215Frameshift
194	2N	535C→T	His179Tyr
259	2N, ↑4N <sup>b</sup>	742C→T	Arg248Trp
294	3.5N	743G→A	Arg248Gln
298	1.8N, 3.1N	836G→A	Gly279Glu
333 <sup>c</sup>	3.4N	633delT	Thr211Frameshift
333	3.5N	637C→T	Arg213Stp
333	3.1N	640delC	His214Frameshift
333	2.4N, ↑4N	659A→G	Tyr220Cys
336 <sup>d</sup>	3.0N	577C→T; 704A→G	His193Tyr; Asn235Ser
355	2.8N	817C→T	Arg273Cys
366	2N, ↑4N	391–393delAAC	Asn131del
391 <sup>e</sup>	2N, 3.4N	916C→T	Arg306Stp
446	3.8N	610–611delGA	Glu204Frameshift
457	3.0N	524G→A	Arg175His
532	2N, ↑4N	944–945insT	Ser315Frameshift
558	3.1N	438G→A	Trp146Stp
592	3.8N	742C→T	Arg248Trp
707	3.4N	818G→T	Arg273Leu
768	2.3N	818G→A	Arg273His
789	2N	388C→T	Leu130Phe
790	2N	743G→A	Arg248Gln
839 <sup>f</sup>	3.7N, ↑4N	650T→G	Val217Gly
839	3.6N	733C→A	Gly245Ser
886	3.1N	586C→T	Arg196Stp
889	3.7N	535C→T	His179Tyr
894	3.2N	524G→A	Arg175His
910	2.4N	743G→A	Arg248Gln
916	2N, 3.7N	743G→A	Arg248Gln
947	2.9N	712T→C	Cys238Arg
980	2N, ↑4N	IVS6+1G→T	Splice donor
994	1.8N	492–493GC→AT	LysGln164LysStp

<sup>a</sup> Ploidy or ploidy with p53 mutation.

<sup>b</sup> ↑4N, 4N fraction >6.0%.

<sup>c</sup> Patient 333 has four different p53 mutations, each in a unique sample.

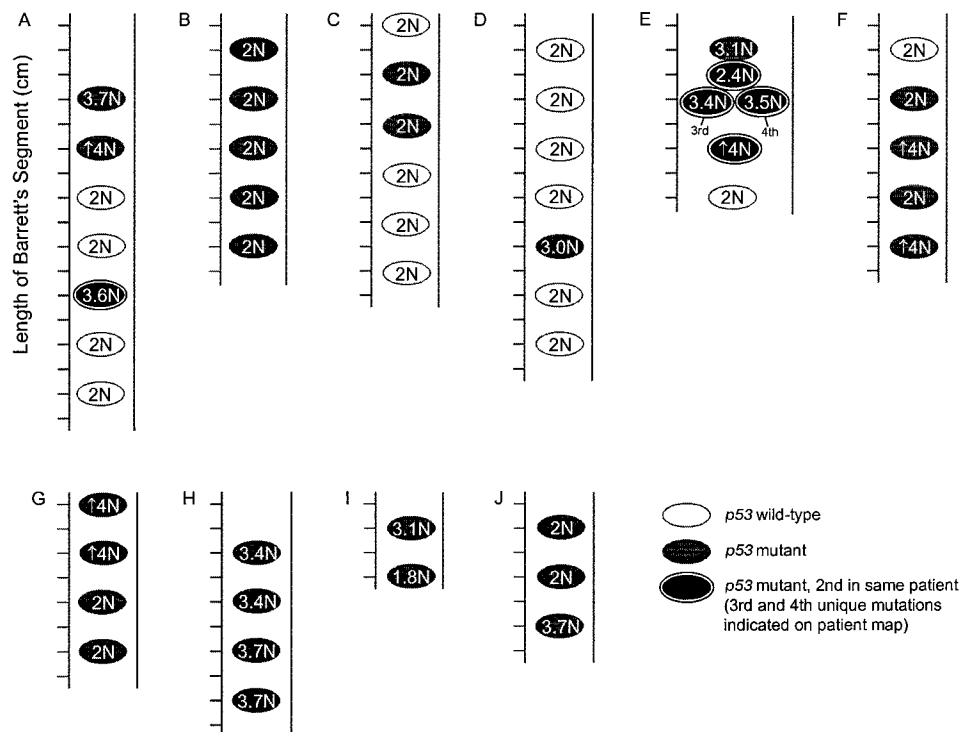
<sup>d</sup> Patient 336 has two different p53 mutations in the same allele.

<sup>e</sup> Mutation reported previously (9).

<sup>f</sup> Patient 839 has two different p53 mutations, each in a unique sample.

<sup>4</sup> B. J. Reid, unpublished data.

Fig. 2. Spatial distribution of p53 mutations in individual patients with high-grade Barrett's esophagus. Representative maps of the location of p53-mutant populations within the Barrett's segment. Exons 5 through 9 of the p53 gene were sequenced using flow-purified cell populations every 2 cm of the Barrett's length. p53 status is indicated by the color in the circle. Ploidy is the number inside the circle. ↑ 4N, a 4N fraction >6.0%. Tick marks, cm. A-J, patient no. and number of centimeters evaluated: A, patient 839, 13 of 17 cm; B, patient 129, 9 of 11 cm; C, patient 790, 11 of 12 cm; D, patient 336, 13 of 15 cm; E, patient 333, 7 of 8 cm; F, patient 980, 9 of 11 cm; G, patient 366, 7 of 8 cm; H, patient 181, 7 of 10 cm; I, patient 298, 3 of 4 cm; J, patient 916, 5 of 7 cm.



field effects is an important question in human neoplastic progression. A field exposure to a carcinogen such as tobacco smoke could result in polyclonal abnormalities (20, 21). Alternatively, mutant cells may spread to create an abnormal clonal field within which multiple cancers can develop (20, 22). The answer to this question will have implications for assessing risk of developing a second cancer, the pathogenesis of the development of second malignancies, and their treatment. Our study of p53 mutations provides evidence for a clonal field effect in the majority of cases and possible multifocal mutagenesis in a smaller number of cases. For example, we observed 12 cases in which a single p53-mutant clone expanded to involve the entire Barrett's segment. In these cases, subsequent somatic genetic abnormalities developing in this p53-mutant clonal field could lead to cancer. Yet, we have also observed three cases in which multiple p53-mutant clones were present in the same patient. Such cases could provide evidence for multifocal carcinogenesis in the Barrett's segment because each clone could have arisen independently and could progress to cancer. We cannot rule out the possibility of a common progenitor in which the p53 mutations developed as subclones. For example, p16 inactivation could precede the observed p53 abnormalities in these cases (9). Regardless, the clonal expansion we have observed for p53-mutant cells has implications for therapy of high-grade dysplasia or early cancer in Barrett's esophagus. These p53-mutant clonal fields may be responsible for the relatively rapid redevelopment of high-grade dysplasia in some patients, as well as the appearance of cancers, after endoscopic ablative therapy (23).

Most of the mutations we detected were either confined to a single level or found throughout the Barrett's segment (Fig. 1). The basis for this distribution is unknown. One possibility is that the mutations arose at different stages in the evolution of the Barrett's segment. Thus, a mutation that developed as an early event might expand as the Barrett's segment expands, whereas a mutation that developed after expansion of the Barrett's segment might be more localized. Alternatively, expansion of p53-mutant clones within the Barrett's segment might require a second hit. Thus, clones without the second hit would remain localized, whereas those with the second hit could expand

rapidly throughout the Barrett's segment. It is unlikely that loss of the remaining p53 allele provides the second hit because 94% of p53-mutant clones in this study had 17p LOH (data not shown).

p53 is inactivated in 50% of human cancers, and it is likely that p53-mutant clonal expansion is common to many human neoplasms. Our observations are consistent with case reports of p53 mutations or 17p LOHs found over extensive areas of other premalignant conditions (20, 24). p53-mutant clonal expansion has been well described in the progression of central nervous systems malignancies (22). Studies of premalignant lung tissue from smokers or ex-smokers have demonstrated extensive expansion of p53-mutant clones as well as multiple independent clones in the same patient in the absence of cancer (20). One study of smokers who developed multiple head and neck cancers reported polyclonal p53 mutations (21). However, other genetic abnormalities, including 3p and 9p LOH, arise as early events during neoplastic progression in upper aerodigestive cancers, and it is possible these or other events represent a common progenitor to the later p53 abnormalities (25). Our results indicate that p53-mutant clones can undergo extensive expansion, creating an abnormal clonal field of mutant cells that frequently involves the entire Barrett's segment. This clonal expansion can occur in diploid p53-mutant clones that have not developed flow cytometric abnormalities or cancer. We also observed multiple distinct p53-mutant clones in a smaller number of cases. Patient 333 was interesting because four p53-mutant clones, each with mutations in exon 6, arose in close spatial proximity. Multiple p53-mutant clones have been described previously in patients exposed to field carcinogenesis (21, 26), and mutation clusters have been reported for the APC gene (27). However, this case was an exception in our study, and the majority of cases provide evidence for simple clonal expansion. Our data are consistent with previous studies indicating that diploid p53-mutant clones are progenitors to neoplastic cell populations with increased 4N fractions, aneuploid DNA contents, and other LOH events (7, 9, 28). Thus, clonal expansion of p53-mutant cells is an early event in neoplastic progression in Barrett's esophagus that creates an abnormal clonal

field in which other genetic abnormalities develop over a period of years before culminating in cancer.

## Acknowledgments

We thank Gary Longton for statistical analysis and Michael Barrett, Thomas Paulson, and David Wong for critical review of the manuscript.

## References

- Devesa, S. S., Blot, W. J., and Fraumeni, J. F., Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer (Phila.)*, **83**: 2049–2053, 1998.
- Cameron, A. J., and Carpenter, H. A. Barrett's esophagus, high-grade dysplasia, and early adenocarcinoma: a pathological study. *Am. J. Gastroenterol.*, **92**: 586–591, 1997.
- Witt, T. R., Bains, M. S., Zaman, M. B., and Martini, N. Adenocarcinoma in Barrett's esophagus. *J. Thorac. Cardiovasc. Surg.*, **85**: 337–345, 1983.
- Neshat, K., Sanchez, C. A., Galipeau, P. C., Blount, P. L., Levine, D. S., Joslyn, G., and Reid, B. J. p53 mutations in Barrett's adenocarcinoma and high-grade dysplasia. *Gastroenterology*, **106**: 1589–1595, 1994.
- Hamelin, R., Flejou, J. F., Muzeau, F., Potet, F., Laurent-Puig, P., Fekete, F., and Thomas, G. TP53 gene mutations and p53 protein immunoreactivity in malignant and premalignant Barrett's esophagus. *Gastroenterology*, **107**: 1012–1018, 1994.
- Gleeson, C. M., Sloan, J. M., McGuigan, J. A., Ritchie, A. J., and Russell, S. E. Base transitions at CpG dinucleotides in the p53 gene are common in esophageal adenocarcinoma. *Cancer Res.*, **55**: 3406–3411, 1995.
- Blount, P. L., Galipeau, P. C., Sanchez, C. A., Neshat, K., Levine, D. S., Yin, J., Suzuki, H., Abraham, J. M., Meltzer, S. J., and Reid, B. J. 17p allelic losses in diploid cells of patients with Barrett's esophagus who develop aneuploidy. *Cancer Res.*, **54**: 2292–2295, 1994.
- Casson, A. G., Tammemagi, M., Eskandarian, S., Redston, M., McLaughlin, J., and Ozcelik, H. p53 alterations in oesophageal cancer: association with clinicopathological features, risk factors, and survival. *Mol. Pathol.*, **51**: 71–79, 1998.
- Barrett, M. T., Sanchez, C. A., Prevo, L. J., Wong, D. J., Galipeau, P. C., Paulson, T. G., Rabinovitch, P. S., and Reid, B. J. Evolution of neoplastic cell lineages in Barrett oesophagus. *Nat. Genet.*, **22**: 106–109, 1999.
- Reid, B. J., Blount, P. L., Rubin, C. E., Levine, D. S., Haggitt, R. C., and Rabinovitch, P. S. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort. *Gastroenterology*, **102**: 1212–1219, 1992.
- Paulson, T. G., Galipeau, P. C., and Reid, B. J. Loss of heterozygosity analysis using whole genome amplification, cell sorting, and fluorescence-based PCR. *Genome Res.*, **9**: 482–491, 1999.
- Kusser, W. C., Levin, D. B., and Glickman, B. W. Sensitive two-stage PCR of p53 genomic DNA exons 5–9. *PCR Methods Appl.*, **2**: 250–252, 1993.
- Gleeson, C. M., Sloan, J. M., McManus, D. T., Maxwell, P., Arthur, K., McGuigan, J. A., Ritchie, A. J., and Russell, S. E. Comparison of p53 and DNA content abnormalities in adenocarcinoma of the oesophagus and gastric cardia. *Br. J. Cancer*, **77**: 277–286, 1998.
- Casson, A. G., Mukhopadhyay, T., Cleary, K. R., Ro, J. Y., Levin, B., and Roth, J. A. p53 gene mutations in Barrett's epithelium and esophageal cancer. *Cancer Res.*, **51**: 4495–4499, 1991.
- Ko, L. J., and Prives, C. p53: puzzle and paradigm. *Genes Dev.*, **10**: 1054–1072, 1996.
- Califano, J., van der Riet, P., Westra, W., Nawroz, H., Clayman, G., Piantadosi, S., Corio, R., Lee, D., Greenberg, B., Koch, W., and Sidransky, D. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res.*, **56**: 2488–2492, 1996.
- Bedi, G. C., Westra, W. H., Gabrielson, E., Koch, W., and Sidransky, D. Multiple head and neck tumors: evidence for a common clonal origin. *Cancer Res.*, **56**: 2484–2487, 1996.
- Slaughter, D. P., Southwick, H. W., and Smejkal, W. "Field cancerization" in oral stratified squamous epithelium. Clinical implications of multicentric origin. *Cancer (Phila.)*, **6**: 963–968, 1953.
- Goldstein, H. M., and Zornoza, J. Association of squamous cell carcinoma of the head and neck with cancer of the esophagus. *Am. J. Roentgenol.*, **131**: 791–794, 1978.
- Franklin, W. A., Gazdar, A. F., Haney, J., Wistuba, I. I., La Rosa, F. G., Kennedy, T., Ritchey, D. M., and Miller, Y. E. Widely dispersed p53 mutation in respiratory epithelium. A novel mechanism for field carcinogenesis [published erratum appears in *J. Clin. Investig.*, **100**: 2639, 1997]. *J. Clin. Investig.*, **100**: 2133–2137, 1997.
- Waridel, F., Estreicher, A., Bron, L., Flaman, J. M., Fontollet, C., Monnier, P., Frebourg, T., and Iggo, R. Field cancerisation and polyclonal p53 mutation in the upper aero-digestive tract. *Oncogene*, **14**: 163–169, 1997.
- Sidransky, D., Mikkelsen, T., Schweddeheimer, K., Rosenblum, M. L., Cavane, W., and Vogelstein, B. Clonal expansion of p53 mutant cells is associated with brain tumour progression. *Nature (Lond.)*, **355**: 846–847, 1992.
- Overholt, B. F., Panjehpour, M., and Haydek, J. M. Photodynamic therapy for Barrett's esophagus: follow-up in 100 patients. *Gastrointest. Endosc.*, **49**: 1–7, 1999.
- Sidransky, D., Frost, P., Von Eschenbach, A., Oyasu, R., Preisinger, A. C., and Vogelstein, B. Clonal origin of bladder cancer. *N. Engl. J. Med.*, **326**: 737–740, 1992.
- Mao, L., Lee, J. S., Fan, Y. H., Ro, J. Y., Batsakis, J. G., Lippman, S., Hittelman, W., and Hong, W. K. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. *Nat. Med.*, **2**: 682–685, 1996.
- Iwamoto, K. S., Mizuno, T., Kurata, A., Masuzawa, M., Mori, T., and Seyama, T. Multiple, unique, and common p53 mutations in a thorotrast recipient with four primary cancers. *Hum. Pathol.*, **29**: 412–416, 1998.
- Nagase, H., and Nakamura, Y. Mutations of the APC (adenomatous polyposis coli) gene. *Hum. Mutat.*, **2**: 425–434, 1993.
- Galipeau, P. C., Cowan, D. S., Sanchez, C. A., Barrett, M. T., Emond, M. J., Levine, D. S., Rabinovitch, P. S., and Reid, B. J. 17p (p53) allelic losses, 4N (G<sub>2</sub>/tetraploid) populations, and progression to aneuploidy in Barrett's esophagus. *Proc. Natl. Acad. Sci. USA*, **93**: 7081–7084, 1996.

# Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

## *p*53-Mutant Clones and Field Effects in Barrett's Esophagus

Laura J. Prevo, Carissa A. Sanchez, Patricia C. Galipeau, et al.

*Cancer Res* 1999;59:4784-4787.

**Updated version** Access the most recent version of this article at:  
<http://cancerres.aacrjournals.org/content/59/19/4784>

**Cited articles** This article cites 28 articles, 9 of which you can access for free at:  
<http://cancerres.aacrjournals.org/content/59/19/4784.full#ref-list-1>

**Citing articles** This article has been cited by 32 HighWire-hosted articles. Access the articles at:  
<http://cancerres.aacrjournals.org/content/59/19/4784.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://cancerres.aacrjournals.org/content/59/19/4784>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.