Frequent Mutation of the E2F-4 Cell Cycle Gene in Primary Human Gastrointestinal Tumors

Rhonda F. Souza, Jing Yin, Kara N. Smolinski, Tong-Tong Zou, Suna Wang, Ying-Qiang Shi, Mun-Gan Rhyu, John Cottrell, John M. Abraham, Kelli Biden, Lisa Simms, Barbara Leggett, G. Steven Bova, Tom Frank, Steven M. Powell, Haruhiko Sugimura, Joanne Young, Noam Harpaz, Kenji Shimizu, Nagahide Matsubara, and Stephen J. Meltzer

Departments of Medicine (GI Division) [R. F. S., J. Y., K. N. S., T. T. Z., S. W., Y. Q. S., J. M. A., S. J. M.], Pathology [J. C., S. J. M.,] and Greenebaum Cancer Center [J. M. A., S. J. M.], University of Maryland School of Medicine and Baltimore Veterans Affairs Medical Center, Baltimore, Maryland 21201-1595; Department of Microbiology, Catholic University School of Medicine, Michigan 48109 (T. F.); Department of Medicine/GI Division, University of Virginia, Charlottesville, Virginia 22908 (S. M. P.); First Department of Pathology, Hamamatsu University School of Medicine, 431-3 Hamamatsu, Japan [H. S.]; Department of Pathology, Mt. Sinai University School of Medicine, New York, New York 10029 (N. H.); and First Department of Surgery and Molecular Genetics, Okayama University Medical School, 700 Okayama, Japan [K. S., N. M.]

Abstract

The E2F family of transcription factors transactivates genes that promote progression through the G1-S transition of the cell cycle. Members of the retinoblastoma (Rb) family of proteins bind to E2Fs and inhibit this function. E2F-4, one example of the E2F group, functions as an oncogene when transfected into nontransformed cells in vitro. On the other hand, mice that are homozygously lacking a normal E2F-1 gene develop cancers, consistent with a tumor-suppressive role for this gene. The exact function of E2F-4 has thus been unclear; moreover, direct involvement of this gene in primary human tumorigenesis has not been shown. We, therefore, investigated mutation within the E2F-4 coding region in 16 primary gastric adenocarcinomas, 12 ulcerative colitis-associated neoplasms, 46 sporadic colorectal carcinomas, 9 endometrial cancers, and 3 prostatic carcinomas. We limited our investigation to the serine repeat within E2F-4, reasoning that this tract might be altered in genetically unstable tumors (replication error-positive, or RER+). All tumors were RER+, with the exception of a control group of 15 RER– sporadic colorectal carcinomas. PCR with incorporation of [32P]dCTP was performed using primers flanking the serine trinucleotide (AGC) repeat. Twenty-two of 59 gastrointestinal tumors (37%) contained E2F-4 mutations; these comprised 5 of 16 gastric tumors (31%), 4 of 12 ulcerative colitis-associated neoplasms (33%, including 1 dysplastic lesion), and 13 of 31 sporadic colorectal cancers (42%). No mutation was present in any of the endometrial, prostate, or RER– colorectal tumors. Of note, homozygous mutations occurred in three cases, and two of seven informative patients showed loss of one E2F-4 allele in their tumors. Furthermore, the RER+ sporadic colorectal tumors were evaluated at trinucleotide repeats within the genes for N-cadherin and B-catenin; no tumors demonstrated mutation of these genes. These data suggest that E2F-4 is a target of defective DNA repair in these tumors.

Introduction

The E2F family of transcription factors has been implicated in the regulation of the cell cycle, particularly at the level of G1-S progression, as well as in the regulation of apoptotic cell death (1–8). E2F-4, one member of this family, is involved in the transition from the resting state (G0) to G1, as well as in the early phase of G1, facilitating the transactivation of genes necessary for cellular proliferation (9). E2F consensus binding sites have been identified in the promoters of several of these growth-regulatory genes, including c-myc, DNA polymerase-α, cyclin-dependent kinases, and cyclin D1 (10–15). E2F-4 is regulated by binding to members of the Rb family of proteins, specifically p130 and p107 (16). In a manner analogous to the interaction of Rb with E2F-1, p130 and p107 inhibit the ability of E2F-4 to transactivate genes while allowing it to retain its DNA-binding capacity (16). In fact, this functional interaction between E2F-4 and p130 suggests distinct mechanisms underlying growth suppression by different Rb family members (17). Regulation of E2F-4 by p130 and p107 is a characteristic of normal growth. However, E2F-4 mutations that prevent its binding to p130 or p107 result in transformation of NIH3T3 cells, which give rise to tumors in nude mice (16). The ability of E2F-4 to transactivate genes, which is normally inhibited by these proteins, is upregulated when E2F-4 is mutated.

Just as the cell cycle is subjected to many regulators (such as E2F-4) that control normal growth and development, the process of carcinogenesis is subjected to the influence of multiple mutational events that interfere with normal growth regulation. One such event is MI. Microsatellites are repeating oligonucleotide tracts present throughout the genome. Mutations within these regions, consisting of the addition or deletion of repeated units, are referred to as the RER+ phenotype, or MI. MI has been demonstrated in many human gastrointestinal tumor types (18–24), usually in noncoding portions of genes; however, it has recently been identified in the protein-encoding portions of two probable tumor suppressor genes, the transforming growth factor-β1 type II receptor and the insulin-like growth factor II receptor (25–29). Interestingly, E2F-4 also contains a polymorphic trinucleotide microsatellite (AGC) repeat, encoding 13 serine residues, within its coding region. We restricted our analysis to the serine repeat region of E2F-4 in a group of RER+ tumors.

Materials and Methods

Tissue Samples. Matching normal and tumor tissues were obtained at the time of surgical resection from 12 patients with UCANs, 46 with sporadic colorectal adenocarcinoma, 16 with gastric carcinoma, 9 with endometrial cancer, and 3 with prostatic carcinoma. The UCANs consisted of 10 carcinomas and 2 dysplastic lesions. All tissues were obtained fresh, dissectedgrossly free of surrounding normal tissue, and frozen in liquid nitrogen.

Received 1/17/97; accepted 5/9/97.

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.

1 Supported by USPHS Grants DK47717, CA67497, and ES07120, American Cancer Society Grant EDT-34525, National Aeronautics and Space Administration Grant 93071-0502, and the Office of Medical Research, Department of Veterans Affairs. R. F. S. is the recipient of National Research Service Award CA68765.

2 To whom requests for reprints should be addressed, at University of Maryland, MedCII, 22 South Greene Street, Room JW362, Baltimore, MD 21201. Phone: (410) 706-3375; Fax: (410) 328-4559.

3 The abbreviations used are: Rb, retinoblastoma; MI, microsatellite instability; RER+, RER–, replication/repair error-positive, -negative, respectively; UCAN, ulcerative colitis-associated neoplasm.
Fig. 1. Alterations of the E2F-4 gene in primary human gastrointestinal tumors. N, normal DNA; T, tumor DNA. Case numbers are shown above each matching normal-tumor pair. Cases AC54, AC58, AC23, AC28, and AC5 are sporadic colorectal carcinomas showing tumor-specific instability of the poly(AGC) tract encoding multiple serines within the E2F-4 protein. In each case, novel alleles are seen in Lanes T that were not present in homologous control Lanes N. Cases SH3, JG831, IG6, and IG15 are gastric adenocarcinomas showing similar abnormalities, whereas case 109 is a colon cancer arising in the premalignant condition, ulcerative colitis. Cases AC44 and AC46 are sporadic colorectal cancers showing homozygous mutation. Only a mutant allele is seen in Lanes T (weak wild-type signal is presumed to originate from contaminating normal cells in the tumor, as well as from the tendency of Taq polymerase to add an extra nucleotide to PCR products). Patients AC56 and AC3 (sporadic colorectal carcinoma) and H33 (an ulcerative colitis-associated colon cancer) are constitutionally heterozygous; two alleles of equal intensity are visible in normal control DNA. Case AC56 does not show loss of heterozygosity; two wild-type alleles of equal intensity are also present in homologous tumor DNA. However, patients AC3 and H33 show loss of heterozygosity; in each case, the upper wild-type allele is absent or markedly diminished in matching tumor DNA.

DNA Extraction. Normal and tumor DNAs were extracted using published protocols (20, 30).

Microsatellite Instability Assays. To verify the RER+ phenotype, DNAs from gastric tumors and UCANs were amplified at microsatellite loci D2S123, D2S147, D2S119, D10S197, and D11S904, which have detected M1 in 67-100% of known RER+ tumors (23). In addition, a subset of gastric tumors was tested at either of two sets of loci: (a) DSS346, D17S804, and p53 intron 1; or (b) D1S116, D6S86, D10S197, D2S136, D17S261, MXS2, and TP53 (23). Sporadic colorectal cancers were tested for M1 using loci MYCL-104, AT3-3I, D2S123, and F13B (31-34) or D2S123, D2S147, D2S119, D10S197, and D11S904. Lesions were classified as RER+ if they manifested instability at one or more of the loci tested. For endometrial tumors, the loci were D2S123, HUMCAJ26, D5S107, D10S197, D11S904, D13S175, D17S1323, and 17q21, which normally encodes 13 serine residues and is located near the 3′ end of the open reading frame.4 Primers used to amplify this region were 5′-TGGTCTCCTGTCCTGGTT-3′, located in the intron upstream of the AGC repeat, and 5′-AAGGAGGTAGAAGGGUGG-3′, located within the coding region of the gene; this PCR yielded a band 310 bp in length. This region was amplified from matching normal and tumor genomic DNAs in separate, independent PCR reactions. Conditions consisted of 35 cycles at 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min. PCR was performed using 0.2 μCi of [32P]dCTP incorporated into a 10-μl reaction mixture. PCR products were denatured in 95% formamide, electrophoresed on 6% denaturing polyacrylamide gels, and visualized by autoradiography. Mutation was defined as a unique band in tumor DNA that was not present in corresponding normal DNA (Fig. 1). Twenty tumors contained E2F-4 alleles one or two codons (3 to 6 nucleotides) shorter than their corresponding normal controls (including one tumor with one to four codons (3 to 12 nucleotides) longer than their corresponding normal controls (including one tumor with both shorter and longer alleles; Table 1). By individual tumor type, 5 of 16 (31%) of the gastric cancers, 4 of 12 (33%) of the UCANs (3 cancers and 1 dysplasia), and 13 of 31 (42%) of the sporadic colorectal carcinomas contained E2F-4 mutations. None of the RER−sporadic colorectal carcinomas had mutation within E2F-4. Results are summarized in Table 1. Furthermore, none of the 31 RER+ sporadic colorectal adenocarcinomas demonstrated mutation within the trinucleotide repeat tracts within the noncoding region of N-cadherin or within the coding region of B-catenin. Finally, none of the nine RER+ endometrial tumors or the three RER+ prostate carcinomas manifested mutation within E2F-4.

In addition to mutations, some tumors also demonstrated allelic loss of E2F-4. The poly(AGC) tract was constitutionally heterozygous in nine patients. Among these nine patients, two demonstrated MI and were deemed invaluable for allelic loss. Of the seven remaining informative patients, two (29%) clearly demonstrated loss of one wild-type E2F-4 allele. Furthermore, an additional three cases dis-
played clearcut homozygous mutation, possessing only mutant E2F-4 alleles in tumor DNAs (Fig. 1).

Discussion

This is the first large-scale report of frequent mutation in primary human tumors within the important cell cycle gene, E2F-4. Previous studies have not reported mutation, whether in microsatellites or in other regions, in additional members of the E2F family (E2F-1, -2, -3, and -5) in primary human tumors; however, one study has also noted mutation of E2F-4 in two primary RER+ colon cancers (36). Mutations affecting the poly(AGC) tract of this gene occurred in 37% of the genetically unstable gastrointestinal tumors and none of the unstable endometrial or prostate carcinomas. Analyzed by specific gastrointestinal tumor type, mutation was detected in 31% of gastric cancers, 33% of UCANs (including one dysplastic lesion), and 42% of sporadic colorectal tumors.

This is the first large-scale report of frequent mutation in primary human tumors. We conclude that mutation of E2F-4 is a frequent and possibly early event in the development of E2F-4 mutation in primary human tumors. We conclude that mutation of E2F-4 is a frequent and possibly early event in the development of gastrointestinal tumors and that E2F-4 mutation in this region is a consequence of defective DNA repair.

References


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