Absence of Genetic Alteration at Codon 531 of the Human c-src Gene in 479 Advanced Colorectal Cancers from Japanese and Caucasian Patients

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Abstract

Activation of c-src, a cellular human gene homologous in sequence to the v-src gene of Rous sarcoma virus, had been thought to play an important role in the progression of several types of human cancers, without having undergone any genetic changes. However, recently truncating mutations at codon 531 of the c-src gene were reported in 12% of the advanced colon cancers, and it was also demonstrated that this change was activating, transforming, tumorigenic, and metastasis promoting. To investigate whether the codon 531-specific mutation could be involved in the carcinogenesis of colorectal cancer in the Japanese and Caucasian populations, we examined a total of 479 advanced colorectal cancers from 421 Japanese patients (46 of them with liver or lung metastases) and from 58 Caucasian patients (11 of them with liver metastases). Using the PCR-RFLP assay and additional single-strand conformation polymorphism analysis, we detected no genetic alteration in any of the advanced colorectal cancers. Our results suggest that the codon 531-specific mutational activation of c-src is unlikely to play a significant role in the malignant progression of colorectal cancers among most Japanese and Caucasian patients.

Introduction

Genetic alterations of proto-oncogenes, such as point mutations observed in the K-ras and H-ras genes, or chromosomal translocations leading to malignant transformation of hematopoietic cells are known to activate oncogenic functions of these genes (1–3). Amplification of some proto-oncogenes (e.g., N-myc, K-sam, and c-erbB-2/HER2) is also known to be associated with specific types of human cancers (4, 5). However, the majority of proto-oncogenes can be transactivated without any apparent genetic changes within the genes themselves. Activated expression of c-src (6), a cellular human gene homologous in sequence to the v-src gene of Rous sarcoma virus, had been thought to have an important role in progression of cancers arising in the colon, breast, liver, pancreas, or head and neck without having undergone any genetic changes (7–9). However, Iry et al. (10) recently documented truncating mutations at codon 531 of the c-src gene in 12% of the advanced colon cancers they tested and also demonstrated that this change was activating, transforming, tumorigenic, and metastasis promoting. Those results provided the first evidence that activating mutations within the c-src gene can play an important role in malignant progression of colon cancer in humans.

To investigate whether codon 531-specific mutation could contribute to the etiology of colorectal cancer in the Japanese and Caucasian populations, we examined a total of 479 advanced colorectal cancers from Japanese and Caucasian patients using the PCR-RFLP assay described by Iry et al. (10) and additional SSCP3 analysis of exon 12 of the c-src gene.

Materials and Methods

Tissue Specimens and DNA Extraction. Primary tumors and corresponding normal tissue samples were obtained from a total of 421 Japanese patients with advanced colorectal cancers, 46 of them with liver or lung metastases, during surgery at the Cancer Institute Hospital (Tokyo, Japan) or the Osaka University Hospital (Osaka, Japan). Samples from 58 Caucasian patients with advanced colorectal cancers, 11 of them with liver metastases, were obtained at the Leiden University Medical Center (Leiden, the Netherlands). Extraction of DNA from these samples was carried out as described elsewhere (11).

PCR Amplification and RFLP Analysis. Analysis of the mutation at position 531 of c-src was performed as described previously (10), with some modifications. The DNAs obtained from 479 colorectal cancers served as templates for each PCR in a thermal cycler (Perkin-Elmer). The primer set was designed to amplify the exon 12, including codon 531 and its adjacent intronic sequences of c-src gene. Examination of the specific codon 531 mutation was performed by digesting the PCR products with a restriction enzyme (SacI); a C-to-T transition would generate a recognition site for SacI at this codon. We amplified placid DNA containing the SRC 531 mutation that generated a SacI site, as a positive control for enzyme digestion.

SSCP Analysis. SSCP analysis was performed to screen the genetic alterations of exon 12 of the c-src gene. A series of SSCP analyses was carried out with a slight modification to the method reported previously (12). Namely, the PCR products used for PCR-RFLP assays were analyzed by electrophoresis, a staining of the gel with fluorescent dye (SyBR Green II, Takara), and a following scan by a Fluorimager (Molecular Dynamics).

Results and Discussion

The discovery of the genetic-activating mutation of src in some cases of advanced human colon cancer in the United States prompted us to examine the c-src gene in primary and metastatic colon cancers resected from 421 Japanese patients, 46 of them with liver or lung metastases. After purifying genomic DNAs derived from paired normal and primary tumor samples and/or distant metastatic tissues, we amplified exon 12 and its adjacent intronic sequences and performed PCR-RFLP assays using a restriction enzyme (SacI) to examine whether codon 531 of c-src had been altered; a C-to-T transition would generate a recognition site for SacI at this codon (10). The PCR-RFLP assays found no mutations at codon 531 in any of the cancer tissues. Furthermore, to exclude the possibility that the specific mutation might have resulted from the ethnic difference between Japanese and Caucasian patients, we examined additional advanced colorectal cancers from 58 Caucasian patients. However, no alteration was found in any of the materials examined (Fig. 1a). Because we did

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3 The abbreviation used is: SSCP, single-strand conformation polymorphism.
identify Scd-digested bands in positive control materials, it is highly unlikely that we missed any C-to-T changes at codon 531. Although we additionally carried out a mutational screening of exon 12 of the c-src gene by SSCP method, we also found no aberrant band, except for positive control (Fig. 1b). In the past several years, some genetic features associated with human cancers, including colorectal cancers, have been extensively studied in ethnically diverse populations; these include activating mutations of the K-ras (13–15) and inactivation of tumor suppressor genes APC (16–18) and/or p53 (19, 20), as well as microsatellite instability (21–23). However, the discrepancy of the frequency of these somatic mutations or replication errors between different ethnic basis was not so significant as that of the c-src mutation we examined. Because we found no mutations at codon 531 in any of the cancer tissues derived from a total of 58 Caucasian patients, the difference on an ethnic basis is unlikely to contribute to this discrepancy.

Considering these results, our failure to detect mutations in our cancer materials could mean that: (a) no c-src mutations were, in fact, present; and (b) activating genetic alterations had occurred in other regions of c-src outside exon 12 in the tumors we studied. According to the method described by Irby et al. (10), the PCR products from tumor samples were sequenced manually, as well as by automated sequencers. In this method, there would be a possible risk for a technical artifact, such as artificial mutation, generated in the process of PCR amplification and for contamination.

c-src and structurally related members of the src family are non-receptor tyrosine kinases that reside within the cell, associated with cell membranes; they seem to transduce signals from transmembrane receptors to the cell interior (24). Many intracellular pathways can be stimulated on src activation, inducing a variety of consequences including morphological changes and cell proliferation (25–27). c-src activity is normally suppressed by phosphorylation on its COOH-terminal region by an enzyme known as COOH-terminal src kinase (28). Various cellular stimuli or disruption of inhibitors may stimulate the endogenous kinase activity of c-src. Previous studies on regulation of src in the chicken had indicated that SRC kinase activity is down-regulated by phosphorylation of a tyrosine at codon 527 in the COOH-terminal region (25, 26). Hence, a truncating mutation at codon 531 of the human homologue was supposed to interfere with phosphorylation of tyr530, the residue equivalent to tyr527 in chicken, and thereby to cause constitutive activation of the c-src protein. In fact, mutational activation of c-src that was found by Irby et al. (10) could lead to a tumorigenic phenotype in vitro, as well as artificially generated mutation in other species (25–27). There is no doubt that studies from several laboratories have documented elevated c-src tyrosine-kinase activity in several types of human primary cancers. Our own observations suggest, however, that mutation at codon 531 of c-src is unlikely to be a predominant mechanism in the malignant progression of colorectal cancers among most Japanese and Caucasian patients and that a different mutational mechanism may be related to activation of c-src tyrosine kinase among advanced colon cancers. To completely clarify the role of c-src activation in human carcinogenesis, future work should examine tyrosine kinase activity, the mutational status of the entire c-src gene, and levels of c-src mRNA and protein expression in tumor samples from both hemispheres and from diverse populations. It is already clear, however, that at least codon 531-specific mutational activation of c-src is not involved in the colorectal carcinogenesis of both Japanese and Caucasian patients we examined.

References

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