Advances in Brief

DNA Copy Number Losses in Chromosome 14: An Early Change in Gastrointestinal Stromal Tumors

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Abstract

The DNA copy number changes were investigated in 13 malignant, 3 borderline, and 16 benign gastrointestinal stromal tumors (GISTs) by comparative genomic hybridization. A consistent finding was the loss of DNA copy numbers in chromosome 14q. This was detected in 75% of the benign tumors, in 62% of the malignant tumors, and in two out of the three borderline tumors with a minimal overlapping region located to 14q22. Losses of DNA copy numbers were more frequent than gains, with 2–10 changes in malignant GISTs and 1–3 changes in benign tumors. High-level DNA amplifications, detected only in malignant GISTs, were assigned to 3q26-q29 (40%), 5p (30%), and 8q22–24 (40%). Our results indicate that copy number losses in 14q are an early change during oncogenesis of GISTs and suggest the possibility that a new tumor suppressor gene at 14q22 might be involved in the regulation of differentiation or proliferation of such mesenchymal cells.

Introduction

Gastrointestinal tract mesenchymal tumors include leiomyomas, schwannomas, and neoplasms composed of uncommitted mesenchymal cells. The last mentioned group is usually designated GISTs.2 Their spectrum encompasses a group of benign, borderline, and malignant tumors as categorized by mitotic activity, cellularity, and tumor size. Immunohistochemical studies have shown GISTs to be characteristically CD34 positive and variably positive for muscle actins but negative for desmin and $\$100$ protein. GISTs are thus different from true leiomyomas, leiomyosarcomas, and schwannomas (1–3).

The genetic changes leading to the initiation and progression of GISTs are entirely unknown. DNA aneuploidy has, however, been demonstrated by flow cytometry in some cases (4).

CGH is a powerful tool for molecular cytogenetic analysis of neoplasms. It enables the screening of entire tumor genomes for gains and losses of DNA copy number and consequent mapping of aberrations to chromosomal subregions (5, 6). To identify genomic areas possibly involved in the oncogenesis of GISTs, we screened DNA copy number changes in whole tumor genomes by CGH. Our most remarkable finding was the frequent loss of DNA copy numbers in chromosome 14q22 both in benign (75%) and malignant (68%) GISTs.

Materials and Methods

Tumor Specimens. The material consisted of 32 GISTs (13 malignant, 3 borderline, and 16 benign). Immunohistochemically defined leiomyomas (desmin positive) and schwannomas ($\$100$ protein positive) were excluded from the study.

The tumors were grouped by the predominant cellular pattern into those with epithelioid or spindle cell morphology. The categorization into benign, borderline, and malignant groups was done on the basis of mitotic counts (benign, less than two mitoses/10 high-power fields; borderline, three to five mitoses/10 high-power fields; malignant, more than five mitoses/10 high-power fields). In addition, tumors with epithelioid morphology over 6 cm in diameter were considered malignant. None of the patients had received chemotheraphy before surgery. Two passages of a cell line established from one case were also studied. DNA from the frozen samples (tumors 1, 2, 5, 17, 20–23, and 29) was extracted according to standard methods, whereas DNA from paraffin-embedded tissues (tumors 3, 4, 6–16, 18–19, 24–28, and 30–32) was extracted as described by Miller et al. (Ref. 7; Table 1).

CGH. CGH was performed using direct fluorochrome-conjugated DNAs for paraffin-embedded samples and an indirect method for frozen samples. Our control studies showed that when DNA from paraffin-embedded tissues was used, the direct method yielded a strong, smooth, and uniform hybridization. With DNA extracted from frozen samples, both methods yielded similar hybridizations. The methods have been described previously (5, 8).

Tumors with DNA copy number changes in chromosomes 1p32–pter, 16p, 19, and 22 were confirmed by another CGH experiment using reversed colors and directly conjugated fluorochromes, because these chromosomal regions have been found to display nonspecific binding of biotin/digoxigenin-labeled DNA used in the indirect method (8, 9).

Direct Method. Tumor DNA was labeled with FITC-dUTP (DuPont, Boston, MA) and reference genomic DNA with Texas red-dUTP (DuPont) using nick translation to obtain DNA fragments ranging from 600 to 2000 bp. The hybridization mixture consisted of the following ethanol-precipitated components: 800 ng of labeled tumor DNA, 800 ng of labeled reference genomic DNA, and 20 μg of unlabeled Cot-1 DNA; the mixture was dissolved in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2× SSC; 1× SSC: 0.15 M NaCl-0.015 M sodium citrate, pH 7). The hybridization mixture was denatured at 75°C for 5 min and hybridized to a slide with normal metaphase spreads denatured in 70% formamide-2× SSC at 70°C for 4 min. Hybridization was performed at 37°C for 2 days followed by slide washes. The slides were washed three times in 50% formamide-2× SSC (pH 7.2), twice in 2× SSC, and once in 0.1× SSC at 45°C followed by 2× SSC and 0.1 M NaH2PO4-0.1 M Na2HPO4-0.1% NP40 (pH 8) and distilled water at room temperature for 10 min each. After air drying, the slides were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (blue) and then mounted with an antifading medium (Vectorshield™, Vector Laboratories, Inc., Burlingame, CA).

Indirect Method. Tumor DNA was labeled with biotin-14-dATP by nick translation using the BioNick™ labeling system (Bethesda Research Laboratories, Bethesda, MD) and reference genomic DNA with digoxigenin-11-dUTP (Boehringer Mannheim Biochemica, Mannheim, Germany). Hybridization conditions were the same as above. Detection was performed using avidin-conjugated tetramethylrhodamine isothiocyanate (red) for biotin-bound DNA fragments, whereas antidigoxigenin FITC was used for digoxigenin-bound DNA. Counterstaining and mounting were performed as above.

Digital Image Analysis. The hybridizations were analyzed using an Olympus fluorescence microscope and the ISIS digital image analysis system (Metasystems GmbH, Altlussheim, Germany) based on an integrated high-sensitivity monochrome charge-coupled device camera and automated CGH.
Reverse transcriptase enzyme adds three to four C residues that are then used to extend the residue on either end of each cDNA molecule. The amplified library is subsequently cloned.

3' end of each cDNA strand beyond the 5' end of each mRNA transcript in a sequence from microdissected cells. mRNA is primed by a modified oligo(dT) primer, and first strand eDNA is synthesized until the 5' end of each mRNA transcript is reached. The poly(A) tailing reaction was performed and the sequences analyzed by BLASTN with the corresponding GenBank database.

Results

The results have been summarized in Table 1. Changes of DNA copy numbers were detected in all GISTs. The most consistent finding, seen both in benign and malignant GISTs, was a loss in DNA copy numbers at 14q. This change was detected in 75% of the benign tumors, in 62% of the malignant tumors, and in two out of the three borderline tumors. The minimal overlapping region of DNA sequence losses was located to 14q22.

Malignant GISTs displayed more changes (12–13; mean, 7) than benign and borderline tumors (1–9; mean, 3). Losses of DNA sequence were more frequent than gains in the benign tumors (85%), whereas gains were equal to losses in malignant tumors. Both passages of the cell line from one case (malignant GIST, case 23) showed the same changes in DNA copy numbers, including those detected in the primary tumor. Several additional DNA changes were detected in the cell line (Table 1).

The most common DNA gains were in chromosomes 5 (25%), 19 (25%), 8 (22%), and 3 (20%) with the minimal overlapping regions 5p, 19q22–qter, and 3q26–qter, respectively. High-level amplifications that were limited to malignant cases were detected at 3q, 3q26–qter, 5p, 5q, 8q22–qter, and 16p. The cell line from case 23 showed additional high-level amplifications at 9q34, 11q22–qter, and 12q22–qter. Fig. 1 presents a summary of common gains and losses of DNA sequences found in 32 GISTs.

In addition to the frequently occurring DNA copy number losses at 14q22, losses were also detected in 1p (28%), 15q (34%), and 22q (34%). The minimal overlapping regions were 1p22-p31.2, 15q21, and 22q12–qter.

Discussion

We studied the cytogenetic changes in GISTs excluding typical leiomyomas and schwannomas. The most striking finding in this study
was the high frequency of loss in DNA copy numbers in chromosome 14 in GISTs. Such losses were also observed in the majority of the benign tumors, in two cases as the only detectable change. The minimal common overlapping area was located to 14q22–qter. This finding suggests that the damage in this part of chromosome 14 is an early change during the tumorigenesis of GIST. LOH at 14q has been reported previously in meningioma (10) and also in ovarian and hepatic carcinoma (11, 12). Together with our results, these findings suggest that a thus-far-unidentified gene with tumor suppressor activity might be present in this region of chromosome 14q.

In many of the GISTs, the loss in 1q4 was accomplished by losses in 1p, 15, and/or 22, which could imply that there is a cumulative effect of deletions of several tumor suppressor genes. Losses of DNA copy numbers on chromosomes 1p, 1q, and 22q have frequently been observed in CGH studies of mesotheliomas, diploid and aneuploid primary breast carcinomas, and gliomas (13, 14). Allele losses on chromosomes 14q and 15q have been reported by LOH in ovarian carcinoma (11) and hepatocellular carcinoma (12), and at 22q in rhabdoid renal tumors (15) and ovarian carcinoma (16). Although the 22q region contains the tumor suppressor gene for neurofibromatosis type 2 (NF2) at 22q12, there is increasing evidence by LOH for another putative tumor suppressor gene distal to NF2 (15). Frequent chromosome losses of 1p have been reported in multiple endocrine neoplasia type 2 (17), hepatocellular carcinoma (18), neuroblastoma (19), breast cancer (20), male germ cell tumors (21), and malignant mesothelioma (22). Deletion in chromosome 1p has been associated with a poor prognosis in neuroblastoma in children (19).

Benign GISTs contained very few gains of DNA copy numbers and no high-level amplifications. In malignant and borderline tumors, DNA copy number gains and high-level amplifications were seen commonly in 3q (38%), 5p (31%), 8q (38%), and Xp (19%). Among the gains at 3q, high-level amplifications were seen at 3q26-ter in two malignant cases and in the cell line. Gains and high-level amplifications were detected also at 5p. Gains at 3q and 5p have previously been seen in small cell lung cancer and in mesothelioma (23), and common observations have been reported in HNSCC (24). The DNA gains and amplifications present in GISTs, especially the changes at 3q and 5p, seem to be associated with a malignant disease. Their possible value as markers of tumor behavior has to be investigated with a larger series of GISTs.

The DNA copy gains and high-level amplifications seen at 8q involved the 8q24 region that contains the proto-oncogene c-Myc known to be amplified in several tumors (25). Gains detected in the X chromosome had the minimal overlapping region and a high-level amplification at Xp which, according to the Genome Data Base, contains several putative target genes that have been implicated in recurrent prostate cancer and osteosarcomas (26). Gains at 18q were seen in four cases (two benign, one malignant, and one borderline). These tumors were histologically of spindle cell type. Because corresponding DNA changes were not observed in other cases, gains at 18q may be associated with GISTs of spindle cell type.

In conclusion, our results show a loss of DNA at 14q22 in both benign and malignant GISTs as the most consistent change. The occurrence of such genomic losses in GIST as well as in previously described mesenchymal tumors suggests that a tumor suppressor gene regulating the proliferation of mesenchymal cells might be located in this area.

References
Genetic Alterations of the Transforming Growth Factor β3 Receptor Genes in Pancreatic and Biliary Adenocarcinomas

Advances in Brief

Pancreatic and Biliary Adenocarcinomas

Departments of Osteology (M. G., C. J. 1., R. H. H., S. E. K.), Pathology (M. S., K. T., R. H. H., S. E. K.), and Surgery (C. J. Y.), Johns Hopkins Medical Institutions, Baltimore, Michael Goggins, Manu Shekher, Kenan Turnacioglu, Charles J. Yeo, Ralph H. Hruban, and Scott E. Kern

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Abstract

Homozygous deletions of ALK-1 gene revealed no alterations. No homozygous deletions were detected in the ALK-1, ALK-2, ALK-3, or ALK-6 genes in a panel of 86 cancer cell lines. The rate of genetic inactivation of one member of this pathway, SMAD4, was identified, but no somatic intragenic mutations were reported in acute myelocytic leukemia (reviewed in Ref. 10), but abnormalities of the ALK-5 gene. Reduced or absent expression of ALK-5 has been demonstrated multiple tumor types (10—13). Inter and PL45, a low-passage cell line established in our laboratory. The TGF-β type I receptor ALK-5 forms a heterodimer with the TGFBR2 genes. Our results indicate that the TGF-β type I and type II receptor-like kinase; RT-PCR, reverse transcription-PCR; LOH, loss of heterozygosity; PRF, polarized retina fibroblast; BMP, bone morphogenetic protein; MAPK, mitogen-activated protein kinase; NTRK, neurotrophic tyrosine receptor kinase; CRK, cytoskeleton-related gene; RHOA, RAS homolog gene; TGF, transforming growth factor; DPC4, deleted in pancreatic cancer 4; MADH4, multiple anaplastic liver staining; TGFBR1, TGFβ receptor type 1; TGFBR2, TGFβ receptor type 2; MAP2, microtubule-associated protein 2; SSAD, Smith—Slatkin—Aburatani—Dean; Bax, proapoptotic Bcl-2 family member; MDR2, multidrug resistance gene 2; SMAD3, SMAD protein 3; SMAD4, SMAD protein 4

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2. To whom requests for reprints should be addressed, at Department of Oncology, 628 N. Wolfe St., Baltimore, MD 21205.

3. The abbreviations used are: TGF-β, transforming growth factor-β; ALK, activin-like kinase; TGFBR1, TGFβ receptor type 1; TGFBR2, TGFβ receptor type 2; MAP2, microtubule-associated protein 2; SSAD, Smith—Slatkin—Aburatani—Dean; Bax, proapoptotic Bcl-2 family member; MDR2, multidrug resistance gene 2; SMAD3, SMAD protein 3; SMAD4, SMAD protein 4

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