Localizations of Chromosome 9p Homozygous Deletions in Glioma Cell Lines with Markers Constituting a Continuous Linkage Group

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Abstract

Southern blot analyses of the 9p-localized type I interferon (IFN) genes in DNAs obtained from malignant glioma cell lines and glioblastoma tissue samples have indicated that homozygous deletions of the IFN-α and IFN-β genes are common during the development of the highly malignant central nervous system neoplasm, glioblastoma. We have applied a set of markers that span the IFN region on 9p to the analysis of DNAs from 30 human glioma cell lines in order to define the region of homozgyous deletion associated with this cancer more precisely. Fourteen of the cell lines revealed either complete (12 cases) or partial (2 cases) homozygous deletions of the IFN-α gene cluster; no instances of homozygous deletions were observed that did not involve the IFN-α region. Genomic DNA identified by the markers nearest to and flanking the IFN-α genes were retained in 5 of the cases with homozygous deletions. Consequently, these results limit the extent of homozygous deletions in glioma cell lines to a small region of 9p21-22 that includes most of the type I IFN locus.

Introduction

The cumulative results of genetic investigations of human glioblastomas and cell lines suggest that numerous TSGs 1 inactivation events are associated with the development of malignant glioma; an inference derived from cytogenetic (1-3) and restriction fragment length polymorphism analyses (4, 5) of such neoplasms which indicate their consistent loss of genetic information at several specific genomic locations. Of these alterations, one of the most common is the karyotypic abnormality involving the structural rearrangement and/or loss of portions of the short arm of chromosome 9 that have been associated with the deletion of the IFN-α and IFN-β gene cluster. Unlike the losses of genetic information which are apparent at other chromosome locations in glioblastomas (e.g., loss of genetic information from chromosome 10; Refs. 4-6), close proximity to the putative TSG involved with the 9p alterations has been suspected because of the frequent detection of homozgyous deletions of the IFN genes (7-9). Such results, in fact, have been interpreted as implicating the type I IFN genes themselves as the target of the 9p alterations.

With regard to the molecular genetic analysis of 9p alterations in malignant glioma, it has been difficult to determine whether the IFN genes are specifically deleted because of the lack of markers which are both localized to the short arm of chromosome 9 and mapped with respect to the IFN locus. This situation has changed as increasing interest has been focused on 9p due to IFN gene deletions and/or 9p rearrangements in a number of human cancers, including melanoma (10), small cell lung carcinoma (11, 12), and leukemia (13, 14), as well as gliomas. The recent presentation of a preliminary genetic and physical map of the interferon region (15) is indicative of this interest, and the markers constituting this map were subsequently used to identify a region of homozgyous deletion centromeric of the IFN-α gene cluster in 2 melanoma cell lines (16). In our report, the same markers have been applied to the Southern blot analysis of a panel of human glioma cell lines, most of which were derived from tumors diagnosed as glioblastomas, to localize the region of 9p deletion and address the specificity of IFN gene loss in the associated cancer. Our results indicate that the critical region is confined by markers nearest to and flanking the IFN-α gene cluster, and that most of the IFN-α gene cluster is within the common region of homozgyous deletion.

Materials and Methods

Cell Lines. Cell lines analyzed in this study were developed at the Cross Cancer Institute (R. S. D.: M006, M007, M016, M021, M027, M035, M040, M0541, M0591, M067, M071, M072, M085K1) and the Karolinska Hospital (V. P. C.: TP265, TP276, TP336, TP365, TP483), or were obtained from Dr. S. A. Aaronson (Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD; cell line A1235), Drs. Paul Kornbluth (Pittsburgh University Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA), and Barry Smith (Cornell Medical College, New York, NY; cell lines RIC, SAN, GRI, and ATCC: U87, U118, U138, U373, HS683, A172, H4, T98G). All except 2 of the cell lines were established from tumors diagnosed as glioblastomas (malignancy grade IV); cell lines HS683 and H4 were derived from neoplasms designated as malignant gliomas (unspecified malignancy grade).

DNA Extraction and Analysis. DNA extractions were performed as previously described (4). Briefly, pelleted cells obtained from culture flasks were lysed in a Tris-C/EDTA/SDS buffer supplemented with 50 μg/ml proteinase K and incubated at 37°C for 3 h. Proteinase K-treated cell lysates were extracted with phenol-chloroform and precipitated with isopropyl alcohol following the addition of ammonium acetate to the DNA solution. Precipitated DNA was resuspended in Tris-EDTA buffer. Resuspended DNAs were treated with restriction enzyme (EcoRI, HindIII, TaqI,MspI, and BanII used in the analysis of each sample), electrophoresed through 0.8 or 0.6% agarose gels, transferred to nylon-based nitrocellulose membranes (NitroPlus, Micro Separations, Inc.) and fixed to the membranes by baking in a vacuum oven at 80°C for 2 h.

DNA filters were hybridized with 32P-labeled probes (random primer technique; Ref. 17) from the following loci: DS93 (2.2-kilobase EcoRI fragment or 2.4 kb HindIII-PstI fragment from clone c5P6; obtained for this study from Dr. J. Fountain, Center for Cancer Research, Massachusetts Institute for Technology, Cambridge, MA), DS919 (4.4-kilobase EcoRI fragment from clone c1RI-L1623; ATCC), DS933 (15-kilobase EcoRI fragment from clone c1RI-494; obtained from Collaborative Research), DS9126 (0.9-kilobase EcoRI subclone, p02-0.9 derived from cosmids c72, Ref. 15; obtained from Dr. F. Fountain), and IFN: both α (624-base pair genomic fragment containing the entire coding sequence; details below) and β (1.9-kilobase EcoRI genomic fragment from clone pBR 13FLOI, Ref. 18; obtained from Y. A. von Gabain, Karolinska Institute, Stockholm, Sweden). The IFN-α probe was amplified from normal, peripheral blood leukocyte DNA by using upstream (AGAGTCAACCATCT-CAGCAAGC) and downstream (ACCACATGTTATCTCCCTCCTC) primers whose sequences correspond to noncoding (upstream) and coding plus noncoding (downstream) genomic DNA that flank the IFN-α “D” gene described...
in Ref. 19. Due to the high degree of sequence conservation between IFN-α genes and pseudogenes, this probe behaves in a manner analogous to other IFN-α probes used for previous Southern analyses (8, 9).

All filters revealing sample nullizygosity for any locus were stripped of probe by soaking filters in 0.4 M NaOH and were rehybridized with additional chromosome 9 probes to demonstrate detectable chromosome 9 DNA in each sample on each filter. Autoradiographic signal response for all instances in which cell line retention of genomic DNA was indicated at any of the investigated loci was quantitated by scanning X-ray films with a Bio-Rad Model 670 imaging densitometer and analyzing resulting images with the associated Molecular Analyst software. Signal responses resulting from hybridization and rehybridization of filters with probes from neighboring loci were normalized and compared as described previously (8).

Results

DNA samples from each of the 30 glioma cell lines were subjected to Southern blot analysis by using probes derived from the D9S3, D9S19, D9S33, D9S126, and IFN loci. Fourteen of these samples were determined to lack DNA from one or more of the corresponding genomic locations (Fig. 1). In all instances where nullizygosity was indicated, filters revealing the homozygous deletions were rehybridized with additional probes from the linkage group to determine whether corresponding genomic DNA for any of the loci were retained in the same tumor cells (e.g., the first three autoradiograms for D9S19, D9S3, and D9S126, as well as the autoradiograms for the two IFN probes in Fig. 1 resulted from hybridization and rehybridization of the same filters). Such analysis revealed that DNA for the markers defining the centromeric and telomeric ends of the linkage group, D9S19 and D9S33, respectively, were retained in all cell lines.

Internal to these points, the probe used for the IFN-α gene revealed the highest number, 14, of homozygous deletions within the panel; no instances of nullizygosity were observed which did not include at least part of the IFN-α gene cluster. Four cell lines (H5683, A1235, M040, and TP265) revealed complete homozygous deletion of the IFN-α gene cluster with retention of DNA for the nearest and flanking markers, D9S126 and IFN-β, while M085K1 displayed complete loss of part of the IFN-α cluster and the centromeric loci D9S126 and D9S3. The results associated with the H4 cell line defined the smallest region of nullizygosity, revealing a homozygous deletion of only part of the IFN-α cluster (Fig. 2). Six distinct regions of nullizygosity were indicated by the 14 cell lines with homozygous deletions, the most common of which involved the entire IFN gene cluster, but neither of the flanking loci, D9S126 and D9S33 (Fig. 2). Although unique regions of homozygous deletion were indicated for only three of the cell lines (H4, SAN, and M085K1), restriction fragment length polymorphism analysis of the loci constituting the linkage group revealed distinct allelic patterns for each member of a specific deletion region (data not shown), thereby establishing that the cell lines with homozygous deletions arose from independent sources.

Autoradiographic signal responses resulting from samples retaining genomic DNA for any of the loci investigated were quantitated and compared with those produced by flanking markers in order to ascertain whether breakpoints within the linkage group, beyond those indicated by points of transition to or from sample nullizygosity, were evident (i.e., normalized cell line signal responses which suggest relative locus copy number transitions of 1 to 2). The results of breakpoints indicated by the identification of nullizygosity and dosage transition points are summarized in Fig. 2.

Discussion

Previous demonstration of the frequent homozygous deletion of the type 1 IFN genes in glioma cell lines has motivated considerable effort toward identification of the genetic target associated with the alteration of chromosome 9p. With regard to this issue, the results of the

Fig. 1. Southern blot analysis of the 9p linkage group in glioma cell line DNAs. EcoRI digests of cell line DNAs were electrophoresed through 0.8 (D9S19, D9S3, D9S126, IFNA, IFNB) or 0.6% agarose gels (D9S33), transferred to nylon-based nitrocellulose membranes, and hybridized repeatedly with probes for the loci indicated on the ordinate. For the D9S19, D9S3, D9S126, IFNB, and D9S33 loci, the restriction fragment displayed represents the only fragment identified by the corresponding probe since each probe was generated and isolated by electrophoresis of an EcoRI digest of the plasmid or phage into which it had been cloned. NC, normal control DNA isolated from peripheral blood leukocytes.

Fig. 2. Summary of regions of homozygous deletion and breakpoints detected in the 9p linkage group. (A) Chromosomal location and (B) genetic distances (centimorgans) between loci investigated in this study (13). (C) Cumulative panel breakpoints between neighboring loci and within the IFN locus as indicated by transitions to and from nullizygosity (B) and by dosage analysis (indicated by Δ) of autoradiograms. (D) Extent of homozygous deletions in cell lines revealing nullizygosity at one or more loci. The IFN locus has been expanded so that breakpoints within the gene cluster can be indicated. The IFN-α gene cluster and IFN-β gene are separated by an arrow and their presumed (22) centromere-telomere orientation is indicated.
present study can be interpreted in at least two ways. The first of these is to view the data as supporting the IFN genes themselves as the objective. In the majority (12 of 14) of instances where homozygous deletions were detected, the entire IFN-a gene cluster was eliminated. Since it has been established that many of the haploid estimate of 20–30 IFN-a genes comprising the cluster are functional (20), it could be that the decrease in IFN protein associated with complete or near-complete homozygous deletions of the cluster would confer tumor growth advantage. In fact, it may very well be that the high frequency of homozygous deletion detected in this tumor and corresponding cell cultures is associated with the inactivation of a gene cluster, rather than a single gene such as p53 or RB (21).

Alternatively, these data can be interpreted in a literal manner as indicating the position of a novel TSG between the D9S126 locus and the telomeric side of the IFN-a cluster (as indicated by the retention of IFN-β in both instances of partial homozygous deletion of IFN-a; Ref. 22). In a similar study of melanoma cell lines (16), a common region of homozygous deletion was determined to involve only the D9S126 locus, thereby indicating the boundaries of this region to be the flanking loci, D9S3 and the IFN gene cluster. As such, there is overlap between the boundaries limiting the region of homozygous deletion in the present study of glioma cell lines with those identified in the melanoma investigation. Consequently, it is tempting to speculate that there is a single TSG whose inactivation is common to the etiologies of both cancers, in spite of the consistent inclusion of the IFN genes in the glioblastoma homozygous deletions.

The two interpretations are not mutually exclusive, however, and it is certainly possible that multiple and distinct 9p TSG inactivation events are important to the etiology of glioma. A resolution of this issue will result from continued physical mapping and characterization of this important region on 9p, as well as by further in vitro and in vivo investigations involving IFN gene replacement in glioma cells (23).

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