Amplification at 12q13–14 in Human Malignant Gliomas Is Frequently Accompanied by Loss of Heterozygosity at Loci Proximal and Distal to the Amplification Site

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Abstract

We have recently reported that a subset of human malignant gliomas shows amplification and overexpression of multiple genes from chromosomal segment 12q13–14, including CDK4, SAS, and MDM2. In the present study we have performed an allelotyping for 16 polymorphic loci spanning both arms of chromosome 12 in a series of 136 gliomas. Allelic deletions were found in 59% (7 of 14) of the malignant gliomas with 12q13–14 amplification and involved loci located on 12q proximal and distal to the amplification site. In contrast, the incidence of allelic loss on chromosome 12 was significantly lower in gliomas without 12q13–14 amplification [14% (11 of 79) in the WHO grade III and IV gliomas, 9% (4 of 43) in the WHO grade I and II gliomas]. The frequent association between 12q13–14 amplification and loss of alleles from 12q is in line with a model suggesting chromosome breakage and deletion as important events in the development of gene amplification.

Introduction

Structural alterations of chromosome 12 have been implicated in the oncogenesis of a number of different human tumor types. Several solid tumors, including lipoma, myxoid liposarcoma, leiomyoma, and pleomorphic adenoma of the salivary glands, are characterized by specific chromosomal translocations with breakpoints clustering at 12q13–15 (1). In addition, amplification and overexpression of multiple genes from 12q13–14 have been shown in certain types of sarcomas (2, 3) and in a subset of malignant gliomas (4, 5). Finally, RFLP studies have revealed frequent allelic deletions on 12q in gastric carcinomas (6) and in male germ cell tumors (7). The majority of the latter tumors are additionally characterized by an increased copy number of 12p (8). Together these cytogenetic and molecular biological studies suggest that chromosome 12 contains a number of tumor suppressor genes and proto-oncogenes, alterations of which seem to be important in the genesis and progression of diverse human tumor types.

In the present study the involvement of chromosome 12 alterations in the molecular pathogenesis of human gliomas was evaluated by performing a comprehensive deletion mapping in a series of 136 such tumors, including 14 malignant gliomas previously shown to have amplification of genes from 12q13–14 (5). Here we report that the incidence of allelic deletions on chromosome 12 in gliomas in general is low. In the group of malignant gliomas with 12q13–14 amplification, however, 50% of the tumors showed allelic loss at loci on 12q.

Materials and Methods

Tumor Material. Paired tumor and blood samples were collected from 136 glioma patients, frozen immediately, and stored at −135°C for up to 4 years. The different glioma types studied are summarized in Table 1. All tumors were classified according to the revised WHO classification of tumors of the central nervous system (9). Histological evaluation of the tumor tissue frozen assured that all specimens studied by RFLP analysis consisted of at least 75% tumor cells.

DNA Extraction and Analysis. Extraction of high molecular weight DNA as well as Southern blotting were carried out as described previously (4, 10). The blots were hybridized with DNA probes labeled with [32P]dCTP by random priming, exposed to phosphor storage screens (Molecular Dynamics, Sunnyvale, USA), scanned in a Molecular Dynamics PhosphorImager, and densitometrically analyzed using ImageQuant software. Locoresearching evidence for allelic loss in the RFLP analysis were additionally studied by quantitative densitometry as described in detail by Reifenberger et al. (10) using the variable number of tandem repeats probe pYNH24 (detects the anonymous locus D2S44) as reference. Locus/gene amplification was considered to be present only when a relative increase in dosage of more than 5 times that of constitutional DNA was found using densitometry and the anonymous reference probe pYNH24 for the D2S44 locus (4, 5). In all cases with amplification, the allelic dosages of several other loci on chromosome 12 were determined by quantitative densitometry to map the amplified region and to exclude polysomy as the cause for the increased signal intensity.

Probes. The following 16 loci on chromosome 12 were analyzed for LOH (chromosomal localization of each locus, probe identity, and restriction enzymes used are given in parentheses): F8WVF (12pter–p12; V8; EcoRI); GLUT3 (12p13.3; pBS-MGTS1; BamHI); D2S125 (12p12.2–p12.1; p12–16; EcoRI); D12S16 (12q12; pTTH101; TaqI); COL2AI (12q2; CosHCol; HindIII); VDR (12q12–q13; pH13; Apal); D12S15 (12q12–q13; pCM1M1.2; TaqI); D12S17 (12q13; pYNH15; MspI); D12S4 (12q13; p9F11; TaqI); D12S14 (12q13; pEFI32.2; MspI); D12S6 (12q13; p11-1-7; MspI); GLI (12q13.3; pK136; EcoRI); D12S8 (12q14–q15; p7G11; MspI); D12S7 (12q21–q23; pDL32B; TaqI); IGFI (12q23; phi61; PvuII); D12S26 (12q24; CRI-L416; EcoRI). The probes for GLI (pKK36P1) was a kind gift from Dr. B. Vogelstein (Baltimore, MD) and the GADDJ53 (CHOP10) probe was kindly provided by Dr. D. Ron (Boston, MA). All other probes including pYNH24 (D2S44) were purchased from the American Type Culture Collection. Generation and characterization of the probes used for MDM2 and CDK4 have been described previously (4, 5).

Results

Among the 136 gliomas studied, 22 tumors (16%) demonstrated LOH at one or more loci on chromosome 12 (Table 1). Quantitative densitometric determination of allelic dosage revealed no evidence for redundancy of the remaining alleles, so that the LOH in all cases is low.
corresponded to simple allelic loss. The incidence of LOH varied among the different tumor types with the highest value obtained for the anaplastic astrocytomas (8 of 23). In the group of glioblastomas, 8 of 47 tumors had LOH on chromosome 12. In contrast, none of 20 WHO grade I and II astrocytomas showed loss of alleles. In the other tumor groups, including oligodendrogial tumors, mixed gliomas, and ependymomas, single cases with allelic losses were found (Table 1).

Only 3 tumors (1 glioblastoma, 1 anaplastic astrocytoma, 1 myxopapillary ependymoma) demonstrated allelic loss restricted to loci on 12p (data not shown). One anaplastic oligoastrocytoma (AOA4) showed LOH at all informative loci on both chromosomal arms (a pattern indicative of monosomy 12), and one anaplastic astrocytoma (AA10) had LOH at all informative loci on 12p and 12q except for the F8VWF locus located at 12pter-p12 (Fig. 1).

In the remaining 17 tumors allelic loss was confined to loci on 12q. Two of these 17 tumors demonstrated LOH at all informative loci from 12q suggesting a deletion of the whole chromosomal arm (GB29 and AA23). Eleven tumors showed patterns indicative of interstitial deletions, three (GB7, GB77, and AA4) with additional terminal 12q deletions. Four neoplasms (GB154, GB130, AA18, and AA20) had LOH at most loci with retention of both alleles at one or two 12q loci (Fig. 1). The commonly deleted region in all 19 tumors with LOH on 12q was located at 12q13–14 and includes the amplified region. Ordinate, analyzed loci and their chromosomal positions (the probes used for RFLP analysis for allelic loss at 16 polymorphic loci located on chromosome 12q13–14 together with loss of alleles at loci distal and/or proximal to the amplification region for CDK4, SAS, and MDM2 are included in the figure to indicate the presence or absence of 12q13–14 amplification (P < 0.01).

Discussion

We have recently reported that about 15% of malignant gliomas of WHO grades III and IV show amplification of DNA sequences derived from the chromosomal segment 12q13–14, including most frequently the genes CDK4, SAS, and/or MDM2 (5). In the present study a representative number of gliomas was investigated by means of RFLP analysis for allelic loss at 16 polymorphic loci located on both arms of chromosome 12. Here we show that allelic loss on chromosome 12 is a relatively rare event in gliomas with high-grade tumors being somewhat more frequently affected than low-grade neoplasms. The notable high value of 35% obtained in the group of anaplastic astrocytomas requires confirmation on a larger number of cases. Our present data suggest that alterations of potential tumor suppressor genes located on this chromosome are of minor significance for the development and/or progression of gliomas in...
suggested in which chromosome breakage and deletion play a central role (13, 14). However, this model is one among many presented to explain the complex processes underlying gene amplification (14, 15). At present, it seems unlikely that gene amplification is caused by a single mechanism. In individual cell types amplification may result from different chromosomal and molecular events. In the case of MYCN amplification in neuroblastomas, for example, it has been demonstrated that amplification generally occurs in the presence of two cytogenetically and molecularly intact chromosomes 2 (MYCN maps to 2p23–24) while the actual amplicons are located either on double minute chromosomes or in homogeneously staining regions located on other chromosomes (16). Recent data obtained by fluorescence in situ hybridization on metaphase chromosomes in well differentiated liposarcomas have revealed a striking association of the 12q13–14 amplicons with ring chromosomes or giant rod marker chromosomes (17, 18). Unfortunately, nothing is known thus far in general. However, it is possible that such alterations might play a role in individual cases. This assumption is supported by the fact that the deletions in gliomas predominantly affect the long arm of chromosome 12 and showed a commonly deleted region at 12q13–14 that corresponds well to results reported for testicular germ cell tumors (7). This commonly deleted region also overlaps with the chromosomal area where translocation breakpoints found in various types of solid tumors are known to cluster (11, 12). Therefore, potential “tumor suppressor genes” are likely to be located in this region. Alteration of one or more of these might provide a growth advantage for the neoplastic cells in individual gliomas.

The high incidence of LOH on 12q in malignant gliomas with amplification of genes from 12q13–14 is striking. In 50% of these tumors the 12q13–14 amplification was accompanied by LOH at one or more adjacent loci. This incidence might increase if more loci around the amplicon region are studied. The molecular basis for this association between amplification and loss on the same chromosomal arm is unclear at present. Experimental evidence suggesting a potential relation between gene amplification and deletion has been provided by studies on drug-induced amplification of the DHFR gene in Chinese hamster ovary cells (13). Chromosome breakage near the DHFR gene and deletion of DHFR and flanking DNA was consistently found in cells that had undergone the amplification process. Based on these results a mechanistic model for gene amplification was...
about the chromosomal or extrachromosomal localization of the 12q13–14 amplicons in glioma cells.

In conclusion, the present study shows that allelic deletions on chromosome 12 occur at low incidences in gliomas. The observed coincidence of 12q13–14 amplification and loss of alleles from 12q is in line with a model in which chromosome breakage and deletion are important events in the development of gene amplification.

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