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Instability of Microsatellites in Human Gliomas[1]

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ABSTRACT

We have analyzed DNA obtained from 10 glioblastomas multiforme and 6 astrocytomas for microsatellite instability, using 17 different microsatellite loci dispersed over 7 different chromosomes.

Six of 16 gliomas showed 1 or more microsatellite alterations in tumor DNA as compared to constitutional DNA. We observed microsatellite instability resulting in allelic shifts in 5 of 10 glioblastomas multiforme but not in any of the astrocytomas. Loss of an allele was observed in 3 glioblastomas multiforme. An imbalance in the intensity of alleles was noticed in 1 astrocytoma and in 1 glioblastoma multiforme. In 1 glioblastoma multiforme, an extra allele was present at two distinct loci. Overall, 5.3% of microsatellite analyses showed an abnormality.

We conclude that microsatellite instability is present at a low grade in glioblastomas multiforme but to a lesser extent in astrocytomas. Genomic instability in human gliomas, therefore, should not be regarded as a mechanism for tumor initiation but as an evolution in tumor progression.

INTRODUCTION

Recently genomic instability has been suggested as a possible mechanism in the development of cancer (1). Initially, this phenomenon was observed in HNPCC[3] as well as in sporadic colorectal tumors (1–6). This instability can be observed as a change in the length of microsatellite sequences in tumor DNA as compared to constitutional DNA. The alterations have been shown to involve di-, tri-, and tetranucleotide repeats. The genomic instability is the result of RER. It has recently been shown that the RER in these HNPCCs is caused by mutations in the *hMSH2* gene, which is located on the short arm of chromosome 2, or by a mutation in the *hMLH1* gene located on the short arm of chromosome 3 (7, 8). These genes are homologous to the bacterial DNA mismatch repair genes. Microsatellite instability has also been found in several other tumor types (9–17). In most tumors, however, genomic instability is reported to be present for only a few microsatellites per tumor. It has therefore been suggested that microsatellite instability, occurring in tumors other than HNPCC, is attributable to processes distinct from those responsible for colorectal cancer. The purpose of this study was to investigate whether genomic instability is present in glial tumors such as astrocytomas and malignant glioblastomas multiforme.

MATERIALS AND METHODS

Sixteen gliomas were obtained from patients treated at the Antwerp University Hospital, from 1991 to 1992, and at the University Hospital in Leuven, Belgium, from 1993 to 1994. Gliomas were classified histologically according to the WHO classification into 10 glioblastomas multiforme, 1 astrocytoma I, 4 astrocytomas II, and 1 astrocytoma III (18). No patient had undergone radiation or chemotherapy prior to tumor resection. Immediately after surgery, the tumor tissue was stored in liquid nitrogen and kept at −80°C. Tumor tissue was cut into small pieces, suspended in a Tris-EDTA buffer containing 0.5% SDS and 500 μg/ml proteinase K, and incubated overnight at 37°C. Tumor DNA was extracted by buffer-saturated phenol/chloroform/isoamyl alcohol, whereas constitutional DNA was isolated using a salting-out method.

Tumor and constitutional DNA from each patient was compared for genetic alterations at 17 different microsatellite loci, located on 7 different chromosomes: chromosome 1 (*MYCL1*); chromosome 5 (*D5S112* and *38.3*); chromosome 8 (*D8S165*); chromosome 11 (*D11S905, D11S956,* and *D11S554*); chromosome 13 (*D13S144, D13S121,* and *D13S134*); chromosome 15 (*CYP19, D15S103,* and *GABRB3*); and the X chromosome (*DXS987, DXS424,* and *P23*). All microsatellites are dinucleotide (CA) repeats, except for microsatellites at *D11S956, D11S554, CYP 19, MYCL1,* and *P23(CTAT)*, which are tetranucleotide repeats, and one microsatellite at *P23 (GTTTT)*, which is a pentanucleotide repeat.

The repeats were analyzed by PCR amplification, followed by electrophoresis on denaturing 6% polyacrylamide gels. Primers (purchased from Eurogentec, Liege, Belgium) and annealing temperatures have been reported before. PCR reactions were performed in a final volume of 20 μl, containing 50 ng of DNA, 4 pmol of each oligonucleotide primer (one labeled at the 5′-end with [γ-^{32}P]ATP and polynucleotide kinase), 200 μmol of deoxynucleotide triphosphate, and 0.5 unit of *Taq* polymerase. After an initial denaturing step at 94°C, 22 cycles at 1 min each were performed at 94°C, 1 min at the optimal annealing temperature, and 1 min at 72°C. A final extension was done at 72°C for 7 min.

RESULTS

Microsatellite patterns at 17 different loci dispersed over chromosomes 1, 5, 8, 11, 13, 15, and X of DNA from a total of 16 gliomas and of DNA from corresponding blood samples were analyzed. The gliomas included 10 GMs and 6 astrocytomas of different malignancy grades (1 grade I, 4 grade II, and 1 grade III). In total, 272 analyses were performed, 264 of which could be interpreted unambiguously.

Differences between normal and tumor DNA were detected in 6 of 16 gliomas (37%), including 5 GMs and 1 astrocytoma. In total 14 microsatellite aberrations were detected. Several types of microsatellite aberrations were found: besides allelic shifts in which the initial number of repeats was increased or reduced, losses of one allele (LOH), the presence of an additional allele, and differences in band density were found. Representative examples of these four different aberrations are shown in Fig. 1.

Four allelic shifts could be demonstrated in 4 different GMs (Fig. 1*A*). A fifth GM showed an extra allele at two different loci, which could be considered as an incomplete shift (Fig. 1*A*, GM5). In total 31% of all analyzed tumors or 1.9% of all analyses showed an allelic shift. In GM5, another shift might be present for *D11S956*, which we interpreted as the loss of heterozygosity (Fig. 1*C*).

In 6 different loci of 1 GM and 1 astrocytoma, we found a clear difference in the band intensity of both alleles. In all six cases, one of the tumor alleles showed a stronger band than did the homologous tumor allele and the two constitutional alleles (Fig. 1*B*). This phenomenon might be explained by a chromosomal duplication or by an amplification of the chromosomal region harboring the locus. The astrocytoma showed this feature only at one locus. The GM (GM5) showed a difference in band intensity at 5 different loci spread over 3 chromosomes, suggesting complex cytogenetic abnormalities.

Three cases of allelic loss were observed in 3 different GMs (Fig. 1*C*).

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[3] The abbreviations used are: HNPCC, hereditary non-polyposis colon carcinoma; RER, replication errors; LOH, loss of heterozygosity; GM, glioblastomas multiforme.

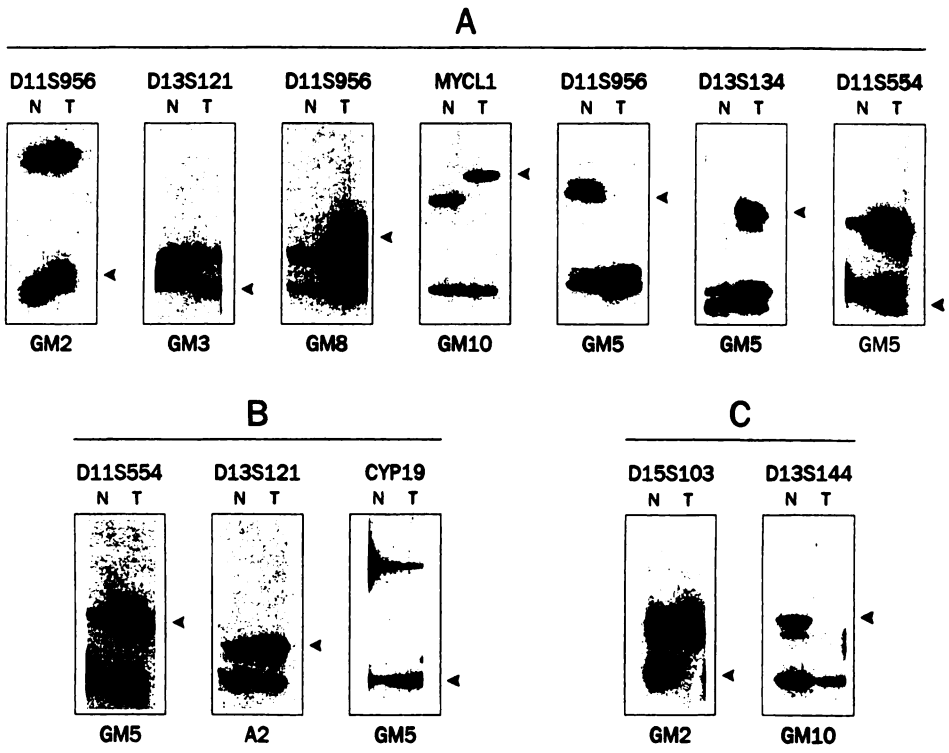

Fig. 1. Aberrations of microsatellites observed in gliomas when comparing normal (*N*) and tumor (*T*) DNA of glioblastomas multiforme (*GM*) and astrocytoma (*A*). *A*, allelic shifts and presence of an extra allele. Tumors GM2, GM3, GM8, and GM10 show an allelic shift in their microsatellite pattern for *D11S956, D13S121, D11S956*, and *MYCL1*, respectively. GM5 shows an extra allele for 2 loci (*D13S134* and *D11S554* and a shift for *D11S956*). *B*, altered allelic band intensity. Tumors GM5 and A2 show differences in band intensity for loci *D11S554, D13S121*, and *CYP19*. *C*, allelic loss. Tumors GM2 and GM10 have lost an allele at loci *D15S103* and *D13S144*, respectively.

In 1.1% of the scored loci, therefore, a mechanism exists which deletes one copy of a microsatellite repeat. It is, however, difficult to state that this is the same phenomenon as classical LOH. In LOH, large chromosomal regions are deleted and sometimes even whole chromosomes are lost, resulting in LOH for that region. Without analyzing loci flanking the repeat showing allelic loss, it is not possible to determine whether the allelic losses we report here are due to LOH of a larger chromosomal region or restricted to a single repeat.

In GM2, however, the predominant alleles seem to be lost in the tumor, retaining one minor allele. Therefore this result can be interpreted as homozygous deletion. In some tumors, the results can be interpreted as loss of heterozygosity of the other allele such as for *D11S554* in GM5, *D13S121* in A2, and *CYP19* in GM5 (Fig. 1*B*).

In total, 14 different microsatellite alterations were found on a total of 264 (5.3%) analyses, with 5 (1.9%) allelic shifts, 6 (2.3%) differences in intensity of homologous alleles, and 3 (1.1%) cases of allelic loss. These alterations were present in 8 dinucleotide as well as in 6 tetranucleotide repeats. To exclude technical artifacts or contamination, all of the differences described were reproduced by independent PCR reactions and separate gel loadings.

DISCUSSION

Our study of human gliomas showed abnormalities of microsatellite repeats which are not present in constitutional DNA from the same patient. In some samples, the interpretation of the aberration was not always obvious, although these problems do not affect the results of this study. Six of 16 (37%) analyzed tumors showed microsatellite instability for at least 1 locus. Nine of 17 (53%) analyzed repeats showed differences for at least 1 tumor. In total, 14 of the 264 (5.3%) analyses showed genomic instability. It is difficult to interpret these findings because there are no data available about the incidence of microsatellite instability in normal brain tissue. The incidence of genomic instability of normal repeats in normal leukocytes is approximately 0.1%, whereas hypervariable repeats have a mutation rate as high as 5% (19, 20). In our series we therefore used only repeats which were not hypervariable. Many recent publications have reported microsatellite instability in a variety of tumors. Unfortunately, the data reported in those studies are hard to compare (Table 1). First, many authors report the percentage of tumors that show microsatellite instability without mentioning the total amount of repeats tested. This is not very relevant inasmuch microsatellite instability can be demonstrated in every tumor when enough microsatellites are tested. Even in leukocytes, "background" microsatellite instability exists. Therefore, it is indispensable to know the percentage of instability not only with respect to the amount of tested tumor samples but also with respect to the total amount of analyses. Second, in many reports it is hard to conclude what the authors mean by microsatellite instability. Microsatellite shifts are to be considered a strong indicator of microsatellite instability, whereas allelic losses, changes in band intensity, and the presence of extra alleles might be indirect indications of genomic instability. Furthermore, an unambiguous interpretation of the results does not always seem to be possible. Third, the number of tumors and/or microsatellite analyses is small in many reports, leading to unreliable estimations of the real incidence of genomic instability. Therefore, we must bear these factors in mind when comparing data from the literature.

A very high percentage of HNPCCs show genomic instability, but only the amount of tumor samples and not the amount of analyses is mentioned in the different reports (Table 1). Nevertheless it is clear from these studies that there is a widespread genomic instability which is much greater than the instability in non-HNPCCs. In the latter tumors, microsatellite instability varies from report to report, even for the same carcinoma (Table 1). This is probably due to the limitations of the studies mentioned above.

In the literature, there is only one study of genomic instability in brain tumors (21). In this study of a variety of brain tumors, including gliomas, genomic instability is reported in 1.8% of tumors and 0.15% of microsatellite analyses. This instability incidence is low and prob-

Table 1 *Review of the literature on microsatellite instability detected in different tumor types*

Tumor type	A[a]		B		Ref.
Adenomas	1/33	(3)[b]			1
Adenomas (HNPCC)[c]	8/14	(57)			1
Adenomas (*MSH2 loss*)[d]	3/4	(75)			1
Bladder cancer[e]	6/200	(3)	15/1400	(1)	9
Brain tumors	1/54	(1.8)	1/648	(0.15)	17
Breast cancer	1/26	(3.8)	1/104	(0.96)	10
Breast cancer	4/20	(20)	13/140	(9.3)	12
Breast cancer	11/104	(10.6)	11/1248	(0.88)	17
Colorectal cancer (sporadic)	40/248	(16.5)			23
Colorectal cancer (sporadic)	8/49	(16)			1
Colorectal cancer (sporadic)	6/46	(13)			1
Colorectal cancer (HNPCC)[c]	11/14	(78.5)			1
Colorectal cancer (HNPCC)[c]	25/29	(86)			1
Colorectal cancer (HNPCC)[c]	9/9	(100)			1
Endometrial cancer (sporadic)[e]	6/36	(17)			11
Endometrial cancer (sporadic)	7/30	(23)			22
Endometrial cancer (HNPCC)[c]	3/4	(75)			11
Endometrial cancer (HNPCC)[c]	4/18	(22)			24
Gastric cancer	16/52	(31)	53/260	(20.4)	12
Stomach (HNPCC)[c]	6/33	(18)			24
Liver cancer	1/29	(3.4)	1/116	(0.86)	10
Ovary cancer	2/20	(10)	2/240	(0.83)	17
Ovary cancer	3/19	(16)	5/77	(6.5)	10
Pancreatic cancer	6/9	(66)	14/33	(42.4)	10
Proximal colon cancer	25/90	(28)			6
Proximal colon cancer	2/18	(11)	5/172	(2.9)	10
Small cell lung cancer	15/33	(45)	55/348	(15.8)	13
Non-small cell lung cancer	13/38	(34)	75/608	(12.3)	21
Soft tissue sarcoma	2/18	(11.1)	2/216	(0.92)	17
Stomach cancer	22/57	(38.5)	43/186	(23)	10
Uterus cancer	2/13	(15)	2/48	(4.2)	10
Glioma[e]	6/16	(37)	15/246	(5.3)	This study

[a] A, Number of tumors revealing microsatellite instability per total amount of tumor samples; B, number of described microsatellite alterations per total amount of analyses.
[b] Numbers in parentheses are percentages.
[c] Tumor associated with HNPCC.
[d] Adenomas in which the DNA mismatch repair gene *MSH2* was lost.
[e] Only in these reports is microsatellite instability defined as "shifts."

ably does not surpass the background instability which is present in every tumor and even in normal tissue. The findings apparently contrast with the incidence of genomic instability in brain tumors found in our study, which is much higher. It should be noticed, however, that the study of Wooster *et al.* (21) is also in contrast with other reports mentioned in Table 1 because the overall genomic instability found by Wooster *et al.* (21) in a variety of tumors is much lower than in other series. We have no obvious explanation for this discrepancy. From our study and the literature, we can conclude that microsatellite instability is a rather common phenomenon in gliomas and in most non-HNPCC tumors. The genomic instability is, however, less frequent and probably due to a phenomenon other than the one descibed for HNPCC.

In 1976, Nowell (22) proposed the clonal expansion model of neoplastic evolution. In his model, he suggested that an initial genetic event starts a previously normal cell to develop neoplastic characteristics. Van de Kelft *et al.* (23) and Cavanee *et al.* (24) have previously suggested that such events may occur in gliomas, with initiation occurring with chromosome 17 deletions in the astrocytoma and progression to glioblastomas multiforme occurring after deletion of chromosome 10. Pathological findings have also demonstrated that malignant gliomas (glioblastomas multiforme) may develop as a result of clonal expansion of an earlier precursor, *i.e.*, astrocytoma (22–24). In our series, genomic instability is more frequently observed in glioblastomas multiforme than in astrocytomas. This might be an indication that genomic instability is not responsible for tumor initiation in gliomas because it cannot be demonstrated in low-grade astrocytomas, but rather it is an indication of tumor progression because it is present in high-grade glioblastomas multiforme.

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