Published OnlineFirst December 5, 2011; DOI: 10.1158/0008-5472.CAN-11-2266

Monoallelic Expression Determines Oncogenic Progression and Outcome in Benign and Malignant Brain Tumors

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Abstract

Although monoallelic expression (MAE) is a frequent genomic event in normal tissues, its role in tumorigenesis remains unclear. Here we carried out single-nucleotide polymorphism arrays on DNA and RNA from a large cohort of pediatric and adult brain tumor tissues to determine the genome-wide rate of MAE, its role in specific cancer-related genes, and the clinical consequences of MAE in brain tumors. We also used targeted genotyping to examine the role of tumor-related genes in brain tumor development and specifically examined the clinical consequences of MAE at *TP53* and *IDH1*. The genome-wide rate of tumor MAE was higher than in previously described normal tissue and increased with specific tumor grade. Oncogenes, but not tumor suppressors, exhibited significantly higher MAE in high-grade compared with low-grade tumors. This method identified nine novel genes highly associated with MAE. Within cancer-related genes, MAE was gene specific; *hTERT* was most significantly affected, with a higher frequency of MAE in adult and advanced tumors. Clinically, MAE at *TP53* exists only in mutated tumors and increases with tumor aggressiveness. MAE toward the normal allele at *IDH1* conferred worse survival even in *IDH1* mutated tumors. Taken together, our findings suggest that MAE is tumor and gene specific, frequent in brain tumor subtypes, and may be associated with tumor progression/aggressiveness. Further exploration of MAE at relevant genes may contribute to better understanding of tumor development and determine survival in brain tumor patients. *Cancer Res; 72(3); 1–9. ©2011 AACR.*

Introduction

LOH of cancer-associated genes is a common and important mechanism in carcinogenesis. However LOH at the level of the RNA, or monoallelic gene expression (MAE), is a much less understood phenomenon. MAE is defined as expression of a gene that is restricted to one allele in the presence of a diploid, heterozygous genome and is observed for imprinted genes and random X inactivation (lyonization). MAE has also been observed in the odorant receptor, immune receptor, and immunoglobulin classes of genes (1, 2). For most other genes, expression is typically expected to occur from both parental alleles without preference. Recent studies, however, have uncovered a potential role for random MAE, as well as differential allele-specific expression (DAE; i.e., a skew in allele-specific expression rather than complete monoallelic expression) in normal tissues as well as in some diseases, including cancer (3–9).

Several studies in normal tissues or derived cell lines have shown random MAE/DAE to be relatively frequent (5%–20%), depending on the number of genes included in the study and the gene selection bias (3, 8, 10). These data together reveal the previously unappreciated random nature of MAE/DAE among nonimprinted genes and provide a mechanism that may help to explain phenotypic differences between individuals, as well as their susceptibility to particular diseases and the disease course.

Cancer is a complex disease, the result of interplay among genetic, epigenetic alterations, and environmental cofactors. We chose brain tumors as a platform to examine MAE for several reasons. Brain tumors are a major cause of morbidity and mortality in pediatric and adult oncology (11). The genetic basis of brain tumors has been studied in depth over the past decade, leading to the identification of mutations/genetic alterations central to brain tumor development (12–15). However, although the role of alterations in the *TP53*, *RB*, and *RAS/MAPK* pathways in the development of specific brain tumors has been well characterized, research considering the widespread impact of MAE in these cancers is in its infancy. Expression of tumor suppressors and oncogenes is tightly regulated during the life of an individual, and it has been established that aberrant gene expression, such as that observed from loss of imprinting, can predispose individuals to cancer development (16). It is therefore reasonable to

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-11-2266

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propose that aberrant gene expression caused by lack of one allele, MAE, could also lead to the development of cancer; indeed preliminary evidence exists to support this notion (7–9).

Previous studies have used Affymetrix and Illumina genome-wide single-nucleotide polymorphism (SNP) arrays to identify MAE in normal and tumor tissues (8, 9), however, the majority of studies investigating MAE/DAE have interrogated either cell lines or germline material from patients, and few have examined primary tumor tissues. Thus the role of MAE/DAE in cancer, and more specifically in brain tumor development, is as yet undetermined. Furthermore, the mechanisms and functional consequences of MAE in primary tumors have not been studied. To examine the role of MAE in brain tumors, we chose specific tumors (pediatric gliomas and choroid plexus tumors) that have distinct clinical and biologic subgroups, benign and malignant, and do not routinely transform from the low- to high-grade state. In addition, we analyzed adult gliomas, as these cancers tend to transform from low- to high-grade and, therefore, represent a clinically mixed group. We also included multiple phases of tumor development, such as autopsy samples, to examine changes in MAE over time. We achieved this by genotyping DNA and RNA (cDNA) samples and identifying SNPs in which DNA genotypes were heterozygous and corresponding RNA genotypes were homozygous. We used Affymetrix SNP 6.0 arrays and high-throughput genotyping techniques to interrogate the extent of MAE and to uncover novel genes displaying MAE. We then examined the role of MAE in specific brain cancer–related genes. We find MAE to be a frequent event in brain tumors; furthermore, it is gene and tumor specific and may precede LOH in mutant genes and predict tumor behavior.

Materials and Methods

Patients and tissues

This study was approved by the Research Ethics Board at The Hospital for Sick Children. Tumor and blood samples from pediatric brain tumor patients were collected from the Pathology Department or through the Arthur and Sonia Labatt Brain Tumor Research Centre at The Hospital for Sick Children. Adult brain tumor tissues were collected from the Canadian Virtual Brain Tumor Bank. The number of tissues analyzed in each group is shown in Table 1. To define the differences between low- and high-grade brain tumors, we analyzed 2 tumor subtypes which are known to have these subgroups: gliomas and choroid plexus tumors. We also compared low- and high-grade gliomas between children and adults, as these exhibit different clinical behaviors. In total, we analyzed 102 samples from 51 tumors by SNP array and 236 brain tumor, blood, and cell line samples (Table 1) for presence of MAE, by genotyping 34 SNPs across 21 candidate genes by Sequenom genotyping (Supplementary Table S1). We also used direct sequencing on a cohort of patients (Table 1) to assess the *TP53* (all exons) and *IDH1* (exon 4) genes for mutations (Supplementary Table S2).

Nucleic acid extraction methods

DNA and RNA were extracted from fresh frozen tumor tissues using standard phenol/chloroform extraction for DNA and TRIzol (Invitrogen) for RNA (see Supplementary Methods for details). The concentration of each nucleic acid was obtained using a NanoDrop spectrophotometer (Thermo Fisher Scientific). In addition, DNA samples were analyzed using a 1% TBE-agarose gel to assess DNA integrity, and RNA samples were analyzed using the Agilent 2100 Bioanalyzer (Agilent Technologies). Nuclear and cytoplasmic RNA were extracted from cell lines using a commercial kit (Norgen Biotek Corporation), following the manufacturers instructions.

Microarray analysis

All nucleic acids were assessed using Affymetrix Human SNP 6.0 arrays (Affymetrix) and all microarrays were

Table 1. Sample composition for the genome-wide analysis, candidate gene analysis, and direct sequencing components

Diagnosis	Analytical method			
	Affymetrix SNP 6.0 arrays	Sequenom genotyping (candidate gene analysis)	Direct sequencing	
			TP53	*IDH1*
pLGG (WHO I/II)	8	61	20	
pHGG (WHO III/IV)	6	27	11	
Pediatric CPP	11		12	
Pediatric CPC	8		17	
Adult low-grade glioma (WHO I/II)	9	30	14	73
Adult high-grade glioma (WHO IV)	9	44	17	71
Blood samples		46	27	
Control normal brain		5		
Cell lines		15	12	
Autopsy brain tumors		8	6	
Total	51	236	136	144

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processed by The Centre for Applied Genomics (TCAG) at The Hospital for Sick Children, Toronto. DNA was processed according to standard Affymetrix protocols. RNA samples were processed according to the GeneChip Whole Transcript (WT) Double Stranded Target Assay protocol, with the following exceptions: (i) the starting amount of RNA was 2 μg and (ii) the cRNA sample generated by the first synthesis cycle was divided into 2 reactions rather than 3, to ensure the volume of double stranded cDNA would be sufficient for hybridization.

Cell culture, media, and drug treatment

Cell lines were maintained as follows: Med8a and D283 cells in DMEM (Dulbecco's modified Eagle's medium; Wisent), UW228, Daoy and fibroblasts 158FB and 1604FB in AMEM (Wisent), D425 and D458 in IMEM with 20% FBS and 1.1 g bicarbonate (Wisent), ONS76 and RES256 (2% FBS) in DMEM/F12 (Wisent) and U87 and U251 in DMEM media (Wisent) with 1% L-glutamine, 1% sodium pyruvate and 1% nonessential amino acids. All media were supplemented with 10% FBS (Invitrogen) unless indicated. Fetal neural stem HF240 cells and glioma tumor-initiating cell lines G179 and GLINS1 were maintained as previously described (17).

Data analysis

Affymetrix Genotyping Console 4.0 was used to genotype the DNA and cDNA samples, using default parameters. Genotypes for untranslated region (UTR) and exonic SNPs were then exported to allow comparison between DNA and cDNA pairs from the same samples. The genotypes for paired gDNA and cDNA samples at each SNP were then compared using a php script, which scored the SNP, depending on whether the gDNA/cDNA genotypes were identical, the cDNA was homozygous when the gDNA was heterozygous, or if there was no result for either gDNA or cDNA. This data was used to determine genome-wide rates of homozygous and MAE genotypes. In addition, gene-based analysis of the SNP data was also carried out for individual samples and groups of samples; genes were only included in this analysis if they were genotyped by at least 2 SNPs. We also compared our overall MAE rates with previously published data about genome-wide predictions of MAE in normal tissues (3). To determine whether the MAE we observed in the genome-wide data was potentially due to allele-specific copy number changes, we examined whether any of our short listed genes were located within regions of copy number change. We found this not to be the case for these samples and genes.

The MassARRAY iPlex system (Sequenom) was used to genotype DNA and cDNA samples. The Sequenom software was used to determine gDNA and cDNA genotypes, and a trained technician then confirmed individual sample genotypes to ensure genotype accuracy. Samples for which the allele ratio was greater than 0.7:0.3 were considered to show differential allelic expression. The Sequenom system is also able to show changes in gDNA copy number; however, we observed no such changes in the SNPs analyzed using this system.

All statistical analyses were done using SPSS v15.

Results

Genomic rate of MAE in brain tumors

To assess the magnitude of MAE in brain tumors in an unbiased manner and to uncover genes that use MAE and contribute to tumorigenesis, we carried out a genome-wide analysis of MAE using Affymetrix SNP 6.0 arrays in a series of 51 low- and high-grade brain tumors (Table 1). For this analysis, we hybridized paired DNA and cDNA from primary tumors to Affymetrix SNP 6.0 chips, to carry out genome-wide genotyping of the DNA and cDNA. To detect MAE, we only considered SNPs for which the DNA genotype was heterozygous, and we removed all homozygous DNA genotypes prior to any analysis. Thus, any cDNA genotype that was subsequently homozygous was considered to display MAE.

To confirm the validity of the Affymetrix SNP 6.0 array in detecting MAE in a genome-wide manner, we examined heterozygous DNA genotypes from the X chromosome of female patients. As expected from the lyonization principle, we observed complete LOH in the cDNA samples, indicating X inactivation (Supplementary Fig. S1). To further validate the utility of this platform to diagnose MAE, we analyzed the frequency of MAE in a subset of imprinted and olfactory receptor genes, which are known to display MAE (1, 2). We observed a median of 82% of informative olfactory receptor genotypes and 50% of informative imprinted gene genotypes were MAE. We confirmed by sequencing the genotypes for SNPs within the *CPA4* gene, which is known to be imprinted, with 100% accuracy.

To determine the role of MAE in low- and high-grade tumors, we investigated the genome-wide rates of DNA homozygosity and MAE within each brain tumor type. As expected, the percentage of SNPs with homozygous DNA genotypes was very high (>80%) in most tumors and higher (albeit not significantly) in high-grade tumors versus low-grade tumors (Table 2). Importantly, for the remaining informative (heterozygote) SNPs, MAE was significantly greater in the pediatric

Table 2. Average percentage of SNPs with homozygous and MAE genotypes

Diagnosis	% homozygous genotypes	% MAE genotypes[a]	*P*[b]
aHGG	88.2	37.7	NS
aLGG	87.3	35.9	
pHGG	89.3	39.5	0.012
pLGG	76.0	28.9	
CPC	90.0	45.4	0.001
CPP	87.7	35.5	

Abbreviations: aHGG, adult high-grade glioma; aLGG, adult low-grade glioma.

[a]The percentage of MAE SNPs, as a proportion of informative genotypes.

[b]*P* value derived from Mann–Whitney test of independent samples for MAE genotypes.

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high-grade gliomas (pHGG) versus pediatric low-grade gliomas (pLGG; $P = 0.012$) and the choroid plexus carcinomas (CPC) versus choroid plexus papillomas (CPP; $P = 0.0011$; Table 2). Genome-wide rates of SNP MAE varied between 28% to 45% of informative genotypes. In addition, the gene-based analysis showed that the number of genes involved in MAE was higher in the pediatric high-grade tumors, though this was only significant in the choroid plexus tumors (CPC vs. CPP, $P = 0.006$; pHGG vs. pLGG, $P = 0.063$, Fig. 1A). Taken together, homozygous expression of alleles (either through DNA LOH or MAE) was greater in pediatric high-grade tumors compared with low-grade tumors, suggesting an important role for MAE in identifying genes involved in the clinical and biologic differences in these tumor subtypes.

In contrast, the frequency of MAE genes was not significantly different between the adult LGG and HGG, which in addition have a very broad range of MAE rates (Supplementary Table S4). This is consistent with the nature of adult gliomas, which invariably progress from low to high grade, whereas pediatric tumors rarely do so.

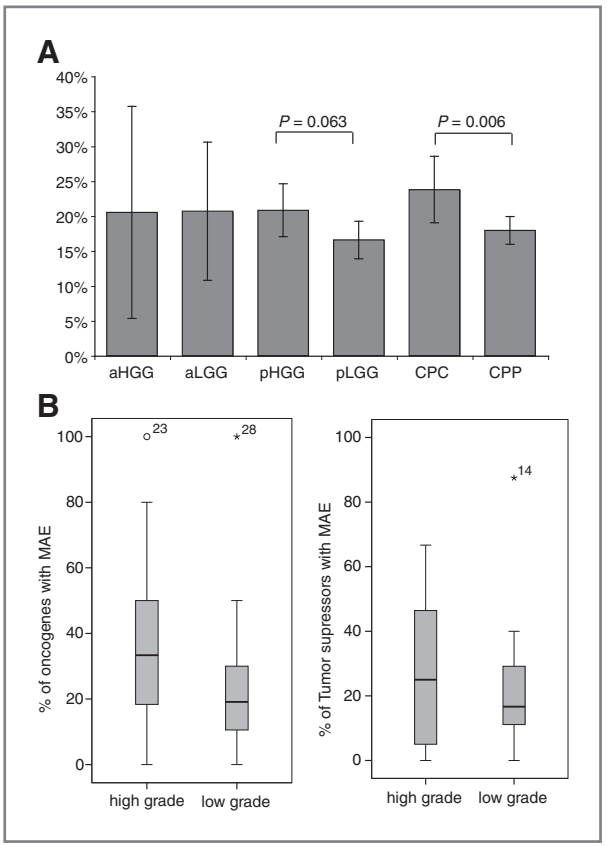

Figure 1. A, average gene MAE in tumor subtypes. The average percentage of genes that have more than 2 SNPs with MAE out of genes with more than 2 heterozygous SNPs is shown for each tumor subtype, ± 1 SD. aHGG = adult high-grade glioma; aLGG = adult low-grade glioma. B, the percentage of known oncogenes and tumor suppressors showing MAE. Box and whiskers plots, in which the box represents the middle 50% of samples, the whiskers show the upper 35% of samples, and the solid lines in the middle of the boxes represent the median value. Data is shown by tumor grade.

As the global, genome-wide MAE rate was greater than the MAE rate observed in the gene-based analysis, we hypothesized that MAE is gene specific. To further investigate this hypothesis, we compared MAE rates in particular gene subgroups (tumor suppressors and oncogenes) and also employed a gene-specific approach to analyze known brain tumor–associated genes (see below).

To determine whether MAE plays a differential role in tumor suppressors versus oncogenes, we investigated the relative frequency of MAE in known tumor suppressors (TS) and oncogenes, as described on the CancerGenes website and via literature searches (Supplementary Table S3). Although the rate of MAE in oncogenes and TS was similar within the low- and high-grade tumor cohorts (Fig. 1B), oncogenes had significantly higher MAE in high-grade tumors than in low-grade tumors (31% MAE vs. 18% MAE, respectively; $P = 0.010$). There was no significant difference in the rate of MAE in tumor suppressors between low- and high-grade tumors ($P = 0.122$). This suggested that high-grade tumors use allele-specific expression of oncogenes during transformation, potentially augmenting mutant or altered alleles.

MAE to detect novel targets

To identify novel tumor-associated genes, we looked specifically at genes with high rates of MAE in each of the tumor types. Using the genome-wide data generated from the Affymetrix SNP 6.0 arrays, we initially restricted our gene list by including only those genes which were informative in more than 75% of tumors within at least one sample group; this yielded a short list of 56 genes. We then identified genes that displayed MAE in 100% of these samples, to identify the genes most affected by MAE. We identified 9 genes that displayed MAE in all informative samples (Table 3). Strikingly, 7 of these 9 genes were reported in the literature to be associated with cancer. Although the proportion of informative cases for these genes in each tumor group varied, all informative samples showed complete MAE at these loci.

Table 3. List of genes with highest rate of MAE in each tumor subtype

Gene	Association with cancer (ref.)
ADAMTS5	Overexpressed in glioblastomas (29)
ST13	Leads to apoptosis via ASK1–JNK pathway (30)
MUC16	Overexpressed in ovarian cancer (31)
ALDH1A2	Candidate tumor suppressor gene in prostate cancer (32)
SLC38A1	Overexpressed in hepatocellular carinoma (33)
TIMELESS	Required for CHK2 activation and checkpoint control (34)
NTRK3	NTRK3 production promotes neuroblastoma cell survival (35)
IYD	Not yet described
TULP4	Not yet described

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Specific analysis of candidate genes reveals high MAE in *hTERT*

Having identified genome-wide differences between different tumor types, we investigated the extent of MAE in known brain tumor–associated genes. To achieve this, we used a semiquantitative genotyping method (Sequenom) to analyze a large cohort of tumor and normal samples for multiple coding SNPs within genes of interest (Supplementary Table S1).

We observed a global increase in MAE/DAE with increasing tumor phase, from normal brain (~7%), through tumors at time of biopsy/resection (~11%), tumors sampled at autopsy (~15%) to cell lines (~27%; Fig. 2A). Interestingly, MAE was observed in only a minority of genes (Fig. 2B) and only 5 genes

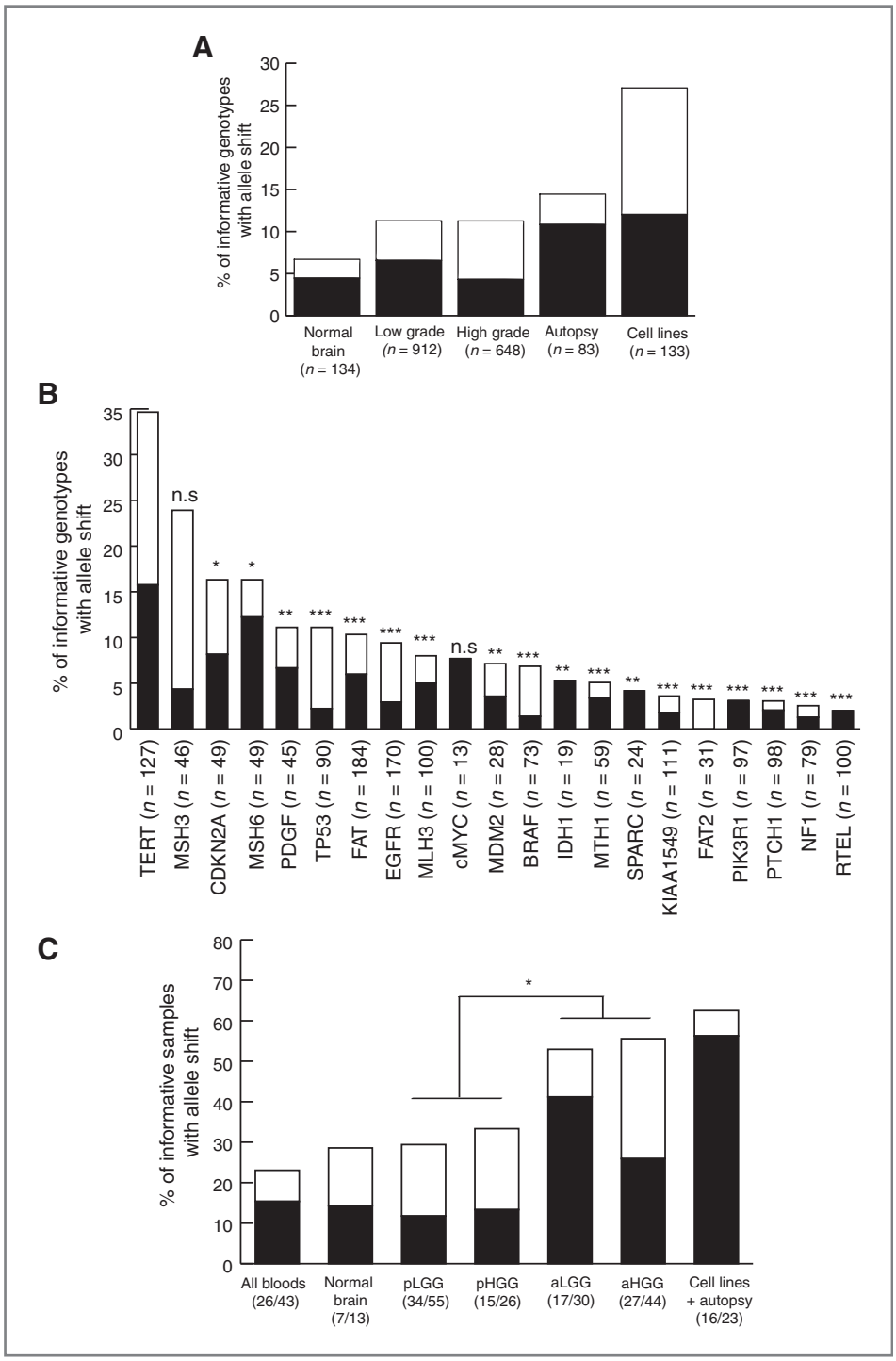

Figure 2. Sequenom analysis of MAE/DAE in normal and malignant brain tissues. Semiquantitative genotyping was carried out on 236 tissues, using 34 SNPs. Because not all SNPs are informative for each tissue, the data is presented as a percentage of the number of informative genotypes within each group. A, rate of MAE/DAE in normal brain, tumors, and cell lines. B, the rate of MAE/DAE at each gene locus. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$. C, the rate of MAE/DAE at the *TERT* locus. *, $P = 0.0224$. For all graphs, the black bars represent MAE, whereas the white bars represent DAE.

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had more than 10% MAE/DAE; *TERT*, the gene with the highest rate of MAE, was recently identified in genome-wide association studies as a risk factor for development of gliomas (18, 19). All other genes, except *MSH3* ($P = 0.2$) and *cMYC* ($P = 0.06$), had significantly lower MAE/DAE rates compared with *TERT* (Fig. 2B).

Higher rate of *hTERT* MAE in adult and advanced brain tumors

The highest rate of MAE/DAE at 35% was observed in *hTERT*. MAE/DAE at *hTERT* varied according to tumor type and phase (Fig. 2C). Specifically, a striking difference was observed between adult and pediatric tumors; more than 50% of adult gliomas showed aberrant *hTERT* expression compared with approximately 30% of pediatric gliomas ($P = 0.0224$, Fisher's exact test). Similarly, the cell line/autopsy tumor samples which represent late phases of tumor evolution had even higher MAE/DAE, in which more than 80% of informative cell lines had aberrant *hTERT* expression. The presence of MAE in the tumors and cell lines was confirmed by direct sequencing. Interestingly, we observed a tendency toward one allele in samples displaying MAE (67% of MAE samples expressed the G allele rather than the A allele for rs2736098); however, there was no difference in allele expression according to tumor grade.

In an attempt to elucidate the mechanisms underlying the observed allele-specific expression at the *hTERT* locus, we first sought to localize the changes in expression, to either pre- or posttranscription. We examined nuclear and cytoplasmic RNA extracts from informative cell lines and found no differences in allelic expression between the 2 cellular compartments, suggesting that the allele-specific alterations in *hTERT* expression were due to pretranscriptional mechanisms.

LOH and MAE of *TP53* correlate with advanced tumor stage

More than 50% of cancers and a significant number of advanced gliomas (20) and CPC (21) harbor either a heterozygous or monozygous somatic *TP53* mutation with LOH. We examined the rate of *TP53* MAE using the common codon 72 polymorphism (rs1042522) and *TP53* mutation status in peripheral blood lymphocytes and brain tumors from different phases (Fig. 3A). We found no MAE in blood samples [including those from Li–Fraumeni syndrome (LFS) patients, who harbor germline heterozygous *TP53* coding mutations]. Furthermore, pLGGs that do not harbor *TP53* mutations did not reveal MAE. In contrast, adult LGGs had an MAE rate of approximately 20%, in addition to the 43% of samples with homozygous *TP53* mutations, resulting in a total of 63% LOH at expressed alleles. For high-grade tumors, which showed the same trend in pediatric and adult samples, approximately 20% of heterozygous samples showed MAE, in addition to the 58% of tumors with homozygous *TP53* mutations, for a total of 78% LOH of expressed alleles. Furthermore, we observed a high rate of homozygous *TP53* mutations in both autopsy tumors (83%) and established long-term cell lines (100%). Finally, to determine whether MAE in a mutant *TP53* tumor could be induced over time, we examined the effect of implanting a *TP53* double heterozygote (c. C455T and G797A) patient-derived glioblastoma cell line into mouse cortex. The xenograft tumor, which previously expressed both alleles, displayed a shift in the allele ratios revealing DAE toward the mutant G797A allele and the normal C455T allele (Fig. 3B). Together, these findings showed association between increasing frequency of homozygous *TP53* mutation at the expression level with increasing glioma grade and disease stage.

The phenomenon of MAE at *TP53* mutation loci was tumor specific because paired blood and tumor DNA/RNA from LFS patients who had MAE in their tumor did not reveal MAE in their blood samples. Furthermore, adult low-grade tumors with MAE predominantly expressed only the normal allele (66% vs. 37% with MAE to the mutant allele), whereas the high-grade tumors predominantly expressed the mutated allele (66% vs. 37% with MAE to the normal allele; data not shown).

To identify the mechanism for MAE at the *TP53* locus, we considered 2 explanations: (i) the presence of promoter polymorphisms that affect transcription factor binding sites, thus leading to allele-specific expression; and (ii) polymorphisms within the 3′-UTR that might affect transcript degradation by altering microRNA attachment, leading to reduced degradation of one allele over the other. We sequenced the reported 2,024 bp of the promoter region (Fig. 3C) in 6 samples with and without MAE. Although we identified a number of known polymorphisms within some of the samples, we did not identify promoter polymorphisms in any MAE sample. We also sequenced the 3′-UTR of known microRNA attachment sites but did not find any mutation or allelic change.

MAE in *IDH1* predicts survival in adult gliomas

We then investigated the role of MAE at another frequently mutated gene in adult gliomas, the *IDH1* (exon 4). We sequenced exon 4 of *IDH1* in an unbiased cohort of 31 adult low-grade tumors and 46 adult high-grade tumors. Consistent with previous data (22), we found a mutation rate of 71% ($n = 22$) in the adult low-grade tumors and 23% ($n = 11$) in the adult high-grade tumors. Of the mutated tumors, 5 (15%) showed MAE. We then investigated the role of MAE at the *IDH1* locus in a second cohort of *IDH1* mutated adult gliomas (42 LGG and 25 HGG), with clinical data available for analysis. Within the clinical cohort, we identified *IDH1* MAE in 12 of 67 tumors (17.9%). In 10 of 12 cases, MAE was specific for the normal allele, which is associated with reduced survival. Clinical correlation within the low-grade glioma cohort revealed significantly higher patient survival ($P = 0.04$) for mutated tumors that did not have MAE, compared with patients whose tumors had MAE toward the normal allele (Fig. 4). This data agrees biologically with previous data, which has shown a survival advantage in patients with mutations, compared with those without *IDH1* mutations (23, 24).

Discussion

Monoallelic expression of genes by allele-specific silencing has been described and is a common mechanism in imprinted genes, immune receptors, and olfactory receptors. The existence of genome-wide random MAE in normal tissues has recently been described (3, 8, 10). However, the

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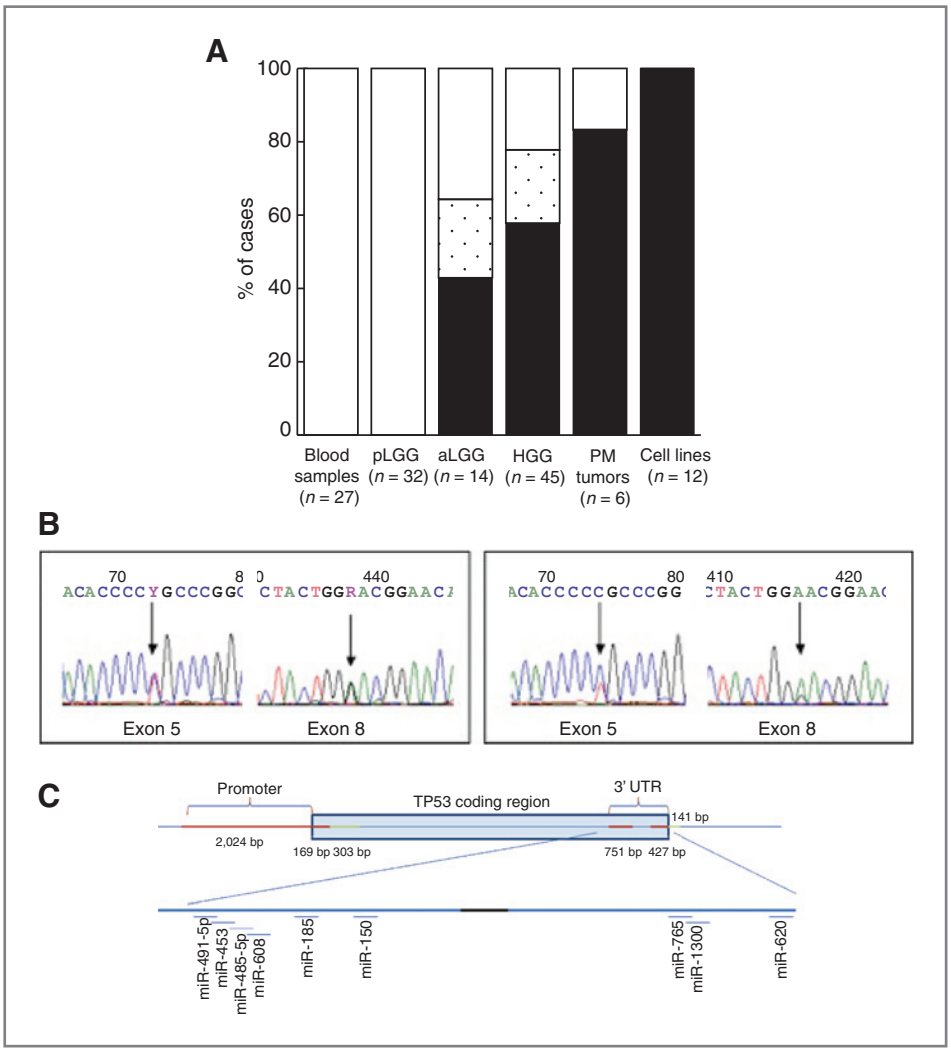

Figure 3. A, progression of *TP53* mutation and monoallelic expression in tumor tissues. This graph shows the percentage of samples tested within each group that have heterozygous *TP53* mutations or heterozygous at codon 72 (solid white), homozygous *TP53* mutations (solid black), or MAE for *TP53* mutations or at the codon 72 polymorphism (pattern). B, allele shift in *TP53* mutations after implantation. GliNS cells were implanted into mice, and the subsequent tumors were analyzed for mutation status. Representative cDNA sequence chromatograms of exon 5 and 8 heterozygous mutations. The panel on the left shows equal allele heights as observed in 3/4 cases, whereas the panel on the right shows representative results from one tumor, which showed a shift in allele expression. This shift was observed in the forward and reverse direction, it was observed for both the exon 5 and exon 8 mutations, and was not observed in the corresponding gDNA samples. C, representative picture of the regions of *TP53* covered by sequencing. At the 5′ end of the gene, we sequenced 2,024 bp 5′ of exon 1, reported to include the entire promoter region, as well as exon 1 and 303 bp of intron 1. At the 3′ end of the gene, we sequenced exon 11, which includes the 3′-UTR, and 141 bp into the on-coding region. We did not sequence 111 bp within the 3′-UTR, as this region contained a large poly-T region but also contained no miRNA-binding sites. miRNA-binding sites predicted using TargetScanHuman (www.targetscan.org/) and microRNA.org (www.microRNA.org).

extent of MAE in brain tumors, and its biologic and clinical significance are still unknown. This study shows that MAE is relatively common in brain tumors. It is a gene- and tumor-specific event rather than a general mechanism and may have a cardinal role in tumor progression, as well as clinical implications.

The extent of MAE in normal tissues ranges from 5% to 20% (3, 8, 10), with approximately 10% of genes showing MAE when analyzed using Affymetrix SNP arrays. This is in agreement with the levels of MAE we observed for most genes in the candidate gene study and the levels of MAE found in blood samples. We observed a continuous trend of low to high MAE from normal tissue, low-grade and high-grade tumors originating from the same tissue, and late established tumor cells, such as autopsy biopsies and cell lines. This finding was consistent on a single-gene level (*TP53* and *hTERT*, Figs. 2C and 3A) as well as on a genome-wide level, in which the average level of MAE within the brain tumor samples was approximately 20% (Fig. 1A) compared with approximately 10% in normal cell lines (3). Furthermore, because DNA LOH increases with tumor progression, the addition of MAE will be associated with a dramatic increase on the rate of allelic expression throughout the process of tumorigenesis. Vogelstein described the process of progression from

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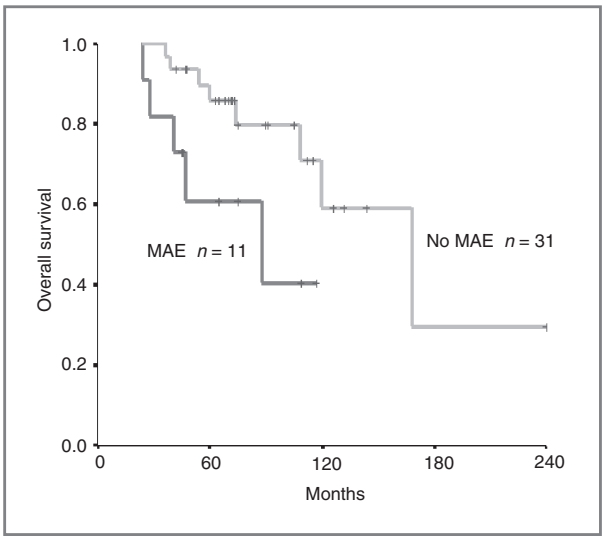

Figure 4. Survival curves for low-grade glioma patients with *IDH1* mutations. All patients had heterozygote (informative) mutations. Patients with *IDH1* mutations and MAE (lower line) to the normal allele had significantly shorter survival than patients with *IDH1* mutations but no MAE (expressing both the mutant and normal alleles, upper line; $P = 0.04$).

premalignant lesions to high-grade tumors in colon cancer through stochastic acquisition of mutations in multiple cancer-related genes (25). Our observations suggest that MAE may precede or enhance a mutation by expression of only the mutant- or disease-related allele and by that may add a new dimension to Vogelstein's prediction.

Our investigation of MAE within specific classes of genes revealed a significant difference in the MAE frequency between low- and high-grade tumors, with fewer oncogenes showing MAE in low-grade tumors. These data suggest that MAE in the context of brain tumors is predominantly advantageous to the tumor, if one considers the biologic situation that predicts that low-grade tumors will have fewer oncogenes disrupted than their high-grade counterparts.

Recent studies have highlighted the potential role for microRNA in cancer development (26, 27), and *TP53* has a number of microRNA binding sites within the 3′-UTR. Although we sequenced the entire promoter, 5′-UTR and 3′-UTR regions, we were able to discard polymorphisms in regulatory regions as the basis for MAE at the *TP53* locus. However, in investigating MAE in the *TP53* gene, we show that MAE can contribute to tumor progression within a single gene. We observed no MAE in tumor samples that do not have *TP53* mutations and that do not progress but observed high rates (~20%) of MAE in tumors that have *TP53* mutations and are progressive. Although one could argue that the mutated transcript is quickly degraded, thus leaving only one allelic form to be detected, we found MAE of both the normal and mutated alleles. Indeed, we observed that low-grade tumors predominantly display MAE of the normal allele, whereas high-grade tumors predominantly express only the mutated allele. These data suggest a tantalizing notion that more malignant tumors are "using" MAE at the RNA level to generate homozygously expressed *TP53* mutations, while still possessing both alleles at the DNA level. This hypothesis agrees with the genome-wide framework, in which we observe more MAE within high-grade tumors compared with nonprogressing low-grade tumors (i.e., pLGGs and CPPs).

The clinical implications of MAE are yet to be realized. Nevertheless, our preliminary findings suggest that MAE can be used to predict survival in tumors harboring mutations that are of prognostic relevance. Our analysis of *IDH1* mutations revealed a clinically significant distinction between mutated MAE tumors and mutated non-MAE tumors. Specifically, mutated *IDH1* gliomas with MAE toward the normal allele have worse survival than non-MAE tumors (Fig. 4). This adds a new dimension to the recent studies which showed that mutations at *IDH1* are predictors of better survival in gliomas (24, 28) and can help tailor therapies for these patients.

In summary, we have shown that in brain tumors, a high level of MAE is present and is tumor and gene specific. Furthermore, MAE may be a mechanism employed by cancers to achieve a more aggressive phenotype. Finally, genome-wide search may identify novel genes involved in these cancers. Additional studies are required to determine the mechanisms that govern MAE and allow for a full appreciation of the role of MAE in disease development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank all of the patients and their families who were involved with the study, as well as the Canadian Virtual Brain Tumor Bank and its affiliate members for the adult tumor tissues and also thank staff at TCAG for assistance and advice with the microarray processing, and staff at AGTC for assistance with processing and analysis of the Sequenom genotype data.

Grant Support

This work was supported by funds received from The Hospital for Sick Children Comprehensive Cancer Centre to U. Tabori, D. Malkin, and E.J. Walker, and in part by funds received from a grant of the Canadian Institutes of Health Research (#MOP-97834) to D. Malkin.

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Received July 7, 2011; revised October 16, 2011; accepted November 21, 2011; published OnlineFirst December 5, 2011.

References

1. Chess A, Simon I, Cedar H, Axel R. Allelic inactivation regulates olfactory receptor gene expression. Cell 1994;78:823–34.

2. Goldmit M, Bergman Y. Monoallelic gene expression: a repertoire of recurrent themes. Immunol Rev 2004;200:197–214.

Published OnlineFirst December 5, 2011; DOI: 10.1158/0008-5472.CAN-11-2266

3. Gimelbrant A, Hutchinson JN, Thompson BR, Chess A. Widespread monoallelic expression on human autosomes. Science 2007;318:1136–40.
4. Frost JM, Monk D, Stojilkovic-Mikic T, Woodfine K, Chitty LS, Murrell A, et al. Evaluation of allelic expression of imprinted genes in adult human blood. PLoS One 2010;5:e13556.
5. Wang J, Valo Z, Smith D, Singer-Sam J. Monoallelic expression of multiple genes in the CNS. PLoS One 2007;2:e1293.
6. Voutsinas GE, Stavrou EF, Karousos G, Dasoula A, Papachatzopoulou A, Syrrou M, et al. Allelic imbalance of expression and epigenetic regulation within the alpha-synuclein wild-type and p.Ala53Thr alleles in Parkinson disease. Hum Mutat 2010;31:685–91.
7. Pinheiro H, Bordeira-Carriço R, Seixas S, Carvalho J, Senz J, Oliveira P, et al. Allele-specific CDH1 downregulation and hereditary diffuse gastric cancer. Hum Mol Genet 2010;19:943–52.
8. Tan AC, Fan JB, Karikari C, Bibikova M, Garcia EW, Zhou L, et al. Allele-specific expression in the germline of patients with familial pancreatic cancer: an unbiased approach to cancer gene discovery. Cancer Biol Ther 2008;7:135–44.
9. Milani L, Lundmark A, Nordlund J, Kiialainen A, Flaegstad T, Jonmundsson G, et al. Allele-specific gene expression patterns in primary leukemic cells reveal regulation of gene expression by CpG site methylation. Genome Res 2009;19:1–11.
10. Pastinen T, Sladek R, Gurd S, Sammak A, Ge B, Lepage P, et al. A survey of genetic and epigenetic variation affecting human gene expression. Physiol Genomics 2004;16:184–93.
11. Porter KR, McCarthy BJ, Freels S, Kim Y, Davis FG. Prevalence estimates for primary brain tumors in the United States by age, gender, behavior, and histology. Neuro Oncol 2010;12:520–7.
12. Dubuc AM, Northcott PA, Mack S, Witt H, Pfister S, Taylor MD. The genetics of pediatric brain tumors. Curr Neurol Neurosci Rep 2010;10:215–23.
13. Nikiforova MN, Hamilton RL. Molecular diagnostics of gliomas. Arch Pathol Lab Med 2011;135:558–68.
14. Sievert AJ, Jackson EM, Gai X, Hakonarson H, Judkins AR, Resnick AC, et al. Duplication of 7q34 in pediatric low-grade astrocytomas detected by high-density single-nucleotide polymorphism-based genotype arrays results in a novel BRAF fusion gene. Brain Pathol 2009;19:449–58.
15. Qu HQ, Jacob K, Fatet S, Ge B, Barnett D, Delattre O, et al. Genome-wide profiling using single-nucleotide polymorphism arrays identifies novel chromosomal imbalances in pediatric glioblastomas. Neuro Oncol 2010;12:153–63.
16. Lim DHK, Maher ER. Genomic imprinting syndromes and cancer. In: Zdenko H, Toshikazu U, editors. Advances in genetics: Academic Press; 2010. p. 145–75.
17. Pollard SM, Yoshikawa K, Clarke ID, Danovi D, Stricker S, Russell R, et al. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. Cell Stem Cell 2009;4:568–80.
18. Shete S, Hosking FJ, Robertson LB, Dobbins SE, Sanson M, Malmer B, et al. Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet 2009;41:899–904.
19. Wrensch M, Jenkins RB, Chang JS, Yeh RF, Xiao Y, Decker PA, et al. Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. Nat Genet 2009;41:905–8.
20. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061–8.
21. Tabori U, Shlien A, Baskin B, Levitt S, Ray P, Alon N, et al. TP53 alterations determine clinical subgroups and survival of patients with choroid plexus tumors. J Clin Oncol 2010;28:1995–2001.
22. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A. Analysis of the *IDH1* codon 132 mutation in brain tumors. Acta Neuropathologica 2008;116:597–602.
23. Kloosterhof NK, Bralten LB, Dubbink HJ, French PJ, van den Bent MJ. Isocitrate dehydrogenase-1 mutations: a fundamentally new understanding of diffuse glioma? Lancet Oncol 2011;12:83–91.
24. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765–73.
25. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
26. Lambertz I, Nittner D, Mestdagh P, Denecker G, Vandesompele J, Dyer MA, et al. Monoallelic but not biallelic loss of Dicer1 promotes tumorigenesis *in vivo*. Cell Death Differ 2010;17:633–41.
27. Huse JT, Brennan C, Hambardzumyan D, Wee B, Pena J, Rouhanifard SH, et al. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis *in vivo*. Genes Dev 2009;23:1327–37.
28. Hartmann C, Hentschel B, Wick W, Capper D, Felsberg J, Simon M, et al. Patients with *IDH1* wild type anaplastic astrocytomas exhibit worse prognosis than *IDH1*-mutated glioblastomas, and *IDH1* mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. Acta Neuropathologica 2010;120:707–18.

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Cancer Res Published OnlineFirst December 5, 2011.