Loss of the AP-2α Transcription Factor Is Associated with the Grade of Human Gliomas

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ABSTRACT

Purpose: **The activator protein (AP)-2α transcription factor plays a crucial role in the progression of several human tumors, including malignant melanoma, prostate, and breast cancer. Loss of AP-2α results in deregulation of several genes with AP-2α binding motifs such as *E-cadherin*, *p21^{WAF1}*, *MMP-2*, *MCAM/MUC18*, *VEGF*, and *c-KIT*. The purpose of our study was to determine AP-2α expression distribution among grades of gliomas and any possible effect on prognosis.**

Experimental Design: **A tissue microarray was assembled from all surgical glioma cases with available tissue samples at M.D. Anderson Cancer Center since 1986 to include 72 glioblastomas, 49 anaplastic astrocytomas, 9 low-grade astrocytoma, 37 oligodendrogliomas, 37 anaplastic oligodendrogliomas, 15 mixed oligoastrocytomas, 20 anaplastic mixed oligoastrocytomas, and 7 gliosarcomas. The microarray included normal brain tissue, and AP-2α expression was determined by immunohistochemistry.**

Results: **AP-2α expression was lost on 99% ($P < 0.001$) and 98% ($P < 0.001$) of glioblastomas and anaplastic astrocytomas, respectively, compared with grade 2 astrocytomas and normal brain, all of which (100%) maintained expression of AP-2α. The loss of AP-2α was a negative prognostic indicator within the overall category of gliomas by univariate analysis (rate ratio, 4.30; 95% confidence interval, 2.60-7.10; $P < 0.001$). However, there was no significant effect of loss of AP-2α expression on survival observed after adjustment for patient age, Karnofsky Performance Scale score, tumor grade, and extent of resection (rate ratio, 1.2; 95% confidence interval, 0.6-2.2; $P = 0.6$).**

Conclusions: **AP-2α expression correlates inversely with glioma grade, suggesting a direct role in glioma tumorigenicity, possibly through subsequent deregulation of target genes. Of all the previously characterized markers of progression, the loss of AP-2α would be the most common (96.2%) molecular marker as an astrocytic tumor evolves from grade 2 to 3.**

INTRODUCTION

Tumor progression depends on factors intrinsic to the tumor cell, including but not limited to growth factors and their cognate receptors, extracellular matrix proteins, proteases, chemokines, and cellular adhesion molecules. The expression of these factors is influenced by the environment, microenvironment, epistasis, and genetic and epigenetic factors. The transcription factor activator protein (AP)-2α has been shown to regulate many of the genes that are involved in normal cellular hemostasis. Therefore, the loss of AP-2α expression may result in dedifferentiation, proliferation, and, eventually, metastasis or invasion.

AP-2α is a 52-kDa protein mapped to the short arm of chromosome 6 near the *HLA* locus (1), a common molecular abnormality found in human astrocytomas. The AP-2α protein binds to a consensus palindromic core recognition element via a DNA-binding domain located within the COOH-terminal half of the protein (2). AP-2α, which is regulated by cyclic AMP and retinoic acid (3), mediates programmed gene expression during both embryonic morphogenesis and adult cell differentiation (4).

AP-2α plays a pivotal role in regulating the expression of several genes, the products of which are involved in tumor growth and metastasis. For example, AP-2α regulates genes that are involved in proliferation, cell cycle regulation (*HER-2* and *p21^{WAF1}*), apoptosis (*c-KIT*, *BCL-2*, and *FAS/APO-1*), adhesion (*MCAM/MUC18* and *E-cadherin*), and invasion/angiogenesis (*MMP-2*, *PAI-I*, *VEGF*, and *PAR-1*). Therefore, loss of AP-2α expression may contribute to functional changes of several gene products that in normal cells are involved in cellular proliferation and differentiation. We have shown previously that down-regulation of AP-2α results in altered *c-KIT* and *MCAM/MUC18* expression, both of which contribute to the increased metastatic potential of human malignant melanoma cells *in vitro* and *in vivo* (5). Results of other studies have provided support for the role played by AP-2α in the progression of mammary and prostate cancer (6, 7).

Studies have suggested that several tumor markers (i.e., *p53*, *EGFR*, and *PDGFR*) may play a role in the transformation of human gliomas. However, these findings have not been consistent, even within WHO tumor grades. The prognostic significance of these tumor markers, especially the most common one, *p53*, is unclear (8–11). *EGFR* amplification has been shown to have prognostic value in patients with anaplastic astrocytoma and in older patients with glioblastoma

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multiforme (12). However, a definite prognostic marker for high-grade gliomas has thus far not been identified. Thus, to determine whether AP-2α expression could be involved in higher-grade gliomas, we intended to determine the level of AP-2α expression across all WHO tumor grades among a large number of human glioma surgical specimens.

MATERIALS AND METHODS

Clinical Samples. Duplicate samples from distinctly different areas of each tumor were arrayed on one slide from resected gliomas with available tissue samples between 1986 and 2001 (n = 246), which included 72 glioblastomas (grade 4), 49 anaplastic astrocytomas (grade 3), 9 low-grade astrocytoma (grade 2), 37 oligodendrogliomas (grade 2), 37 anaplastic oligodendrogliomas (grade 3), 15 mixed oligoastrocytomas (grade 2), 20 anaplastic mixed oligoastrocytomas (grade 3), and 7 gliosarcomas (grade 4) archived in paraffin blocks. Included on this slide were normal brain tissue (positive control) and glioma cell lines (negative control). Cross-referencing to each patient's medical chart was possible through use of the Department of Neurosurgery clinical database and pathology records. Survival time was defined as the time from the M.D. Anderson Cancer Center surgery to death or to the most recent follow-up.

Microarray Immunohistochemistry. Sections obtained from the archived paraffin blocks were deparaffinized and steamed in Serotec target unmasking fluid (Serotec, Inc., Raleigh, NC) for antigen retrieval. Samples were blocked with endogenous peroxidase with 3% H_2O_2 followed by blocking in 4% fish gelatin and PBS.

Samples were incubated overnight with primary antibody diluted in blocking solution. AP-2α was detected using a 1:1,000 dilution of anti-AP-2α polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Competition studies with a blocking peptide (Promega, Madison, WI) showed that this polyclonal antibody was specific for AP-2α (13, 14). The primary antibody was washed with PBS and incubated with a biotinylated secondary antibody. Streptavidin-horseradish peroxidase was added followed by 3-amino-9-ethylcarbazole. The samples were counterstained with Gill's no. 3 hematoxylin and subsequently washed with water and mounted with universal mount. Samples were considered AP-2α positive when two blinded, independent investigators verified positive nuclear staining of the arrayed tissue by microscopic evaluation. In almost all cases, positive AP-2α staining was uniform and virtually 100% of the tumors cells were positive. In the only exception to this rule, the mixed tumors, we determined a specimen was positive when >50% of the cells were positive.

Vascular endothelial growth factor (VEGF) was detected using a 1:1,000 dilution of anti-VEGF antibody (Santa Cruz Biotechnology) and matrix metalloproteinase (MMP)-2 was detected using a 1:1,000 dilution of anti-MMP-2 antibody (Chemicon International, Temecula, CA). The primary antibody was washed with PBS and incubated with the appropriate dilution of biotinylated secondary antibody. Slides were washed again with PBS and exposed to horseradish peroxidase-avidin complex, washed, and developed with diaminobenzidine (ImmunoPure Ultra-Sensitive ABC Staining kit, Metal Enhanced DAB Substrate kit, Pierce, Rockford, IL). The specimen was considered to have increased expression when the amount of horseradish peroxidase staining was clearly at least 2-fold greater than normal brain tissues arrayed on the same slide.

Statistical Analysis. The Fisher exact test was used to evaluate differences in the various characteristics between AP-2α-positive and AP-2α-negative groups as well as the relative frequency of AP-2α positive (compared with low-grade glioma) among the different glioma histologies and grades. Kaplan-Meier estimates of survival were generated, and univariate and multivariate analyses of predictors of survival were done using the Cox proportional hazards model. Crude and adjusted rate ratios and their 95% confidence intervals (95% CI) were obtained. $P < 0.05$ was considered significant.

RESULTS

Study Population. The median age of the patients was 42 years (range, 4-82 years). The majority of patients (97%) had a Karnofsky Performance Scale score of ≥70. Seven patients had a biopsy. Among the remaining 239 patients, the median extent of resection was 96%. The AP-2α-positive group was significantly younger (median, 38 years; range, 4-64 years) compared with the AP-2α-negative group (median, 44 years; range, 4-82 years; $P < 0.001$). The AP-2α-positive group had a lower extent of resection (median, 89%; range, 19-100%) compared with the AP-2α-negative group (median, 99%; range, 12-100%; $P = 0.008$).

AP-2α Expression Is Lost within High-grade Gliomas. In the vast majority of the samples, determination of intranuclear AP-2α positivity was absolute and usually uniform, although exceptions occurred, especially in the mixed tumors such as the mixed oligoastrocytoma, in which the distribution was patchy. In the mixed oligoastrocytoma and the anaplastic mixed oligoastrocytoma cases, those cells with astrocytic features (fibrillary processes) were virtually 100% devoid of AP-2α and those cells with oligodendroglioma features (uniformly round regular nuclei with a classic "fried egg appearance") were uniformly positive. In these cases, when >50% of the intranuclear staining were positive for AP-2α expression, the specimen was determined to be positive (Fig. 1).

Normal brain, including both white and gray matter and the cerebellum, had ubiquitous expression of intranuclear AP-2α. Among the nine specimens from patients with astrocytoma, 100% maintained expression of AP-2α. Among the 49 specimens from patients with anaplastic astrocytoma, only 2% maintained expression of AP-2α ($P < 0.001$ compared with astrocytoma), and among the 72 glioblastoma specimens, only 1% still maintained expression ($P < 0.001$ compared with astrocytoma). In the case of the 37 oligodendrogliomas, 78% of the specimens maintained positive expression of AP-2α. However, in the 37 anaplastic oligodendroglioma specimens, only 35% of the samples still maintained expression of AP-2α ($P = 0.0004$ compared with oligodendroglioma). In the 15 tumors that had mixed oligoastrocytoma features, 80% maintained positive expression, but again as the histologic grade became more anaplastic (n = 20), only 60% maintained positive expression. Although this difference was not statistically significant, it supports the trend observed with anaplastic astrocytoma versus

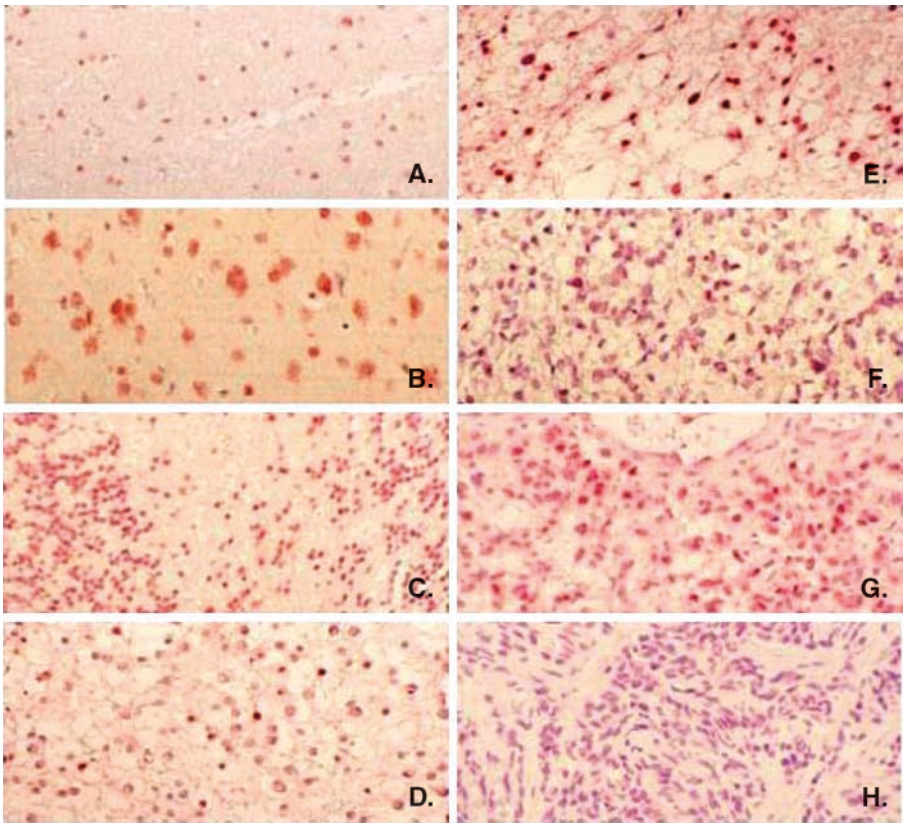

Fig. 1 AP-2α expression within human gliomas as detected by immunohistochemical staining with anti-AP-2α on archival tissue arrays. Tissues were considered positive for AP-2α expression when two blinded, independent observers confirmed positive nuclear staining. *A,* normal white matter demonstrating positive AP-2α staining; *B,* normal neurons (gray matter) demonstrating positive AP-2α staining; *C,* normal cerebellum demonstrating positive AP-2α staining; *D,* typical AP-2α-positive oligodendroglioma; *E,* typical AP-2α-positive fibrillary astrocytoma (grade 2); *F,* typical anaplastic astrocytoma (grade 3) that did not stain positive for AP-2α; *G,* the only glioblastoma multiforme that maintained expression of AP-2α; *H,* typical glioblastoma multiforme (grade 4) that did not stain positive for AP-2α. Original magnification of all images, ×200.

astrocytoma and mixed oligodendroglioma-astrocytoma versus oligodendroglioma. In the case of gliosarcoma ($n = 7$), 29% still had positive expression of AP-2α ($P = 0.005$; Table 1).

AP-2α Expression Is Lost within High-grade Astrocytomas Compared with High-grade Oligodendrogliomas. Among WHO grade 3 and 4 tumors, there was a significant difference in AP-2α expression in anaplastic astrocytoma compared with anaplastic oligodendroglioma ($P < 0.0001$), suggesting that AP-2α has a greater roll in the progression of astrocytic-type tumors. Among WHO grade 2 tumors, there was no significant difference in AP-2α expression in astrocytoma versus oligodendroglioma ($P = 0.32$). Thus, AP-2α seems to be a marker of grade 3 and 4 astrocytic gliomas.

AP-2α Expression Affects Downstream Markers. There was a statistically significant association of AP-2α loss with overexpression of MMP-2 and VEGF. For example, in astrocytoma, none had lost expression of AP-2α and none overexpressed MMP-2 or VEGF ($P < 0.0001$). However, in glioblastoma, in which 99% had lost expression of AP-2α, 31% and 28% of the tumors overexpressed MMP-2 and VEGF, respectively ($P < 0.0001$). This statistically significant trend was observed across all grades for VEGF overexpression. Similar findings were seen with MMP-2 expression, except in anaplastic oligodendroglioma and anaplastic mixed oligoastrocytoma, in which the loss of AP-2α was not significantly associated with overexpression of MMP-2 ($P = 0.63$ and 0.34, respectively).

Table 1 AP-2α expression within gliomas among WHO grades

Histology (WHO grade)	*n*	AP-2α expression, *n* (%)	95% CI*	*P*†
Low-grade astrocytoma (2)	9	9 (100)	66-100	___
Oligodendroglioma (2)	37	29 (78)	62-90	0.32
Mixed oligoastrocytoma (3)	15	12 (80)	52-96	0.27
Anaplastic astrocytoma (3)	49	1 (2)	0-11	<0.001
Anaplastic oligodendroglioma (3)	37	13 (35)	20-53	<0.001
Anaplastic mixed oligoastrocytoma (3)	20	12 (60)	36-81	0.03
Glioblastoma (4)	72	1 (1)	0-7	<0.001
Gliosarcoma (4)	7	2 (29)	4-71	0.005

NOTE: A tissue array was established that contained specimens of resected tumors (encompassing all WHO grades) from patients with gliomas. The expression of AP-2α was determined by immunohistochemistry.

*For the proportion with AP-2α expression.

†As determined by Fisher's exact test in comparison with low-grade astrocytoma.

AP-2α Expression Affects Survival. As high as 109 of 167 patients with loss of AP-2α died during follow-up. The Kaplan-Meier estimate of median survival for patients in this group was 1.64 years (95% CI, 1.14-2.14; Fig. 2). Only 18 of 79 patients with AP-2α-positive tumors died during follow-up. The loss of AP-2α was a negative prognostic indicator within the overall category of gliomas by univariate analysis (rate ratio, 4.30; 95% CI, 2.60-7.10; $P < 0.001$; Table 2), however, that effect was lost after adjustment for important confounding variables such as age, Karnofsky Performance Scale, extent of surgical resection, and tumor grade (adjusted rate ratio for loss of AP-2α, 1.18; 95% CI, 0.63-2.20, $P = 0.62$). No significant differences in the rates of survival between the two AP-2α groups were seen within each tumor grade. For example, in the only glioblastoma patient whose tumor expressed AP-2α, the survival time was 19.9 months, which was not significantly different from the 18-month median survival time of the remaining AP-2α-negative-expressing glioblastoma patients. In the case of the two patients with anaplastic astrocytoma who maintained AP-2α expression, the mean survival time was 49.3 months, which compared favorably with the 35-month mean survival time in the other patients with anaplastic astrocytoma, whose tumors were AP-2α negative. However, these groups of patients are too small to allow meaningful statistical analysis. Patients with oligodendroglioma, whose tumors maintained AP-2α expression, had a mean survival time of 47.8 months, which again compared favorably with the 34.3-month survival time in patients whose oligodendroglioma were AP-2α negative, but this was not statistically significant. No significant difference was seen in mean survival time between patients with AP-2α-positive anaplastic oligodendroglioma and those with AP-2α-negative anaplastic oligodendroglioma (51 versus 50 months, respectively). In the case of the 15 patients with mixed oligoastrocytoma, a trend toward longer survival time was seen in the 12 patients whose tumors maintained AP-2α expression compared with the three patients whose tumors were AP-2α negative (60.8 versus 47.9 months, respectively). No significant differences were seen in mean survival time between patients with AP-2α-positive and those with AP-2α-negative anaplastic oligodendroglioma (47.8 versus 46.5 months, respectively).

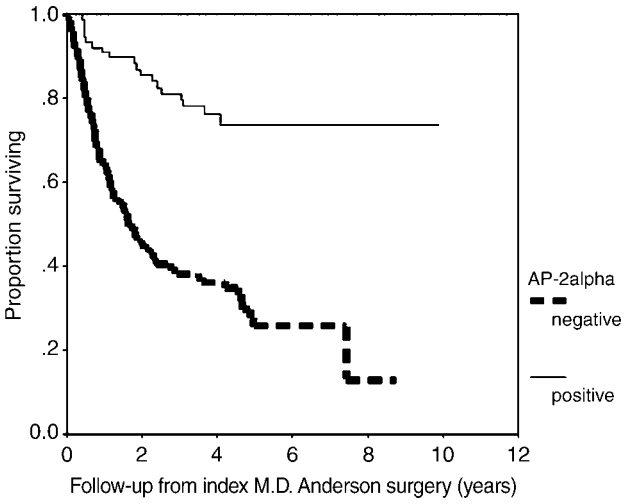

Fig. 2 Loss of AP-2α is a negative prognostic indicator among all glioma patients. Median survival for patients without AP-2α expression was 1.6 years (95% CI, 1.1-2.1 years), which is in marked contrast to those patients who still maintained AP-2α expression (median not reached; $P < 0.0001$). However, AP-2α is not a prognostic indicator within histologic subtypes or tumor grades or after adjustment for known prognostic indicators.

DISCUSSION

Using tissue microarrays, we have shown for the first time that high-grade human gliomas are associated with the loss of expression of the AP-2α transcriptional factor, which regulates several genes, such *MCAM/MUC18*, *MMP-2*, and *c-KIT* that have been shown to be important for tumor progression and invasion. Over time, lower-grade astrocytomas can acquire genetic mutations and subsequently evolve to a higher grade (secondary glioblastoma). Differential genetic pathways leading to primary and secondary glioblastoma have been elucidated. The most well-characterized tumor markers associated with progression of glioblastoma are *p53*, *MDM2*, *EGFR*, and *PDGF*. The frequency of p53 protein accumulation in secondary glioblastoma is >90%, whereas in primary glioblastoma expression is <35%. *MDM2* forms a complex with p53, thus abolishing its transcriptional activity. Immunohistochemical staining of overexpression of *MDM2* is observed in >50% of primary glioblastomas but in <10% of secondary glioblastomas. *EGFR* is overexpressed in ~30% to 60% of the primary glioblastoma but rarely in secondary glioblastoma (10%). Finally, *PDGF* overexpression is present in ~60% of secondary glioblastoma (15). The expression of AP-2α strongly correlates with tumor grade and there is a ubiquitous loss between grade 2 and 3 astrocytomas. Therefore, of the previously characterized tumor markers, the loss of AP-2α would be the most common (96.2%) molecular marker as an astrocytic tumor evolves from grade 2 to 3.

Based on the loss of heterozygosity and dosage analysis in human astrocytoma, the second most common genetic abnormality was identified previously on chromosome 6p (16). Additionally, allelic imbalance on 6p was present in 57% of patients with astrocytoma (17). The transcriptional factor AP-2α has been mapped to human chromosome 6p22 (1). The discrepancy between the data from the previously published cytogenetic analyses and our tissue microarray data may be because of different sensitivities of these assays. Point mutations resulting in a nonfunctional protein would not be detected by cytogenetic analysis. Ultimately, comparisons of *in situ* hybridization for AP-2α with 6p alterations in the same tumor to show correlation will ultimately be necessary to definitely establish this relationship.

We have shown previously that transfection of melanoma cells lines with a dominant-negative AP-2β, which inhibits AP-2 transactivator function, results in an increase in MMP-2 expression, activity, and subsequent invasion (18). Additionally, overexpression of AP-2α in prostate cell lines resulted in down-regulation of VEGF. AP-2α was found to repress VEGF by competing with the transcriptional activator Sp3. Loss of AP-2α in prostate cancer cells increased VEGF expression (19). Similar to these previous findings, we found that gliomas that lost AP-2α were more likely to overexpress VEGF and/or MMP-2 compared

Table 2 Effect of AP-2α on survival: all patients

Characteristics	Univariate analysis			Multivariate analysis	
	Median, y (95% CI)	Rate ratio (95% CI)	*P*	Rate ratio (95% CI)	*P*
AP-α status					
Negative	1.64 (1.14-2.14)	4.30 (2.60-7.10)	<0.001	1.18 (0.63-2.20)	0.62
Positive*	NR	1.00	—	1.00	—
Age (y)	—	1.04 (1.01-1.06)	<0.001	1.03 (1.01-1.04)	<0.001
Karnofsky Performance Scale					
<70	0.76 (0.54-0.98)			3.28 (1.16-9.24)	0.025
>70	4.05 (2.53-5.58)	1.00	—	1.00	—
Histology					
Glioblastoma/gliosarcoma grade 4	1.07 (0.68-1.46)	15.58 (7.38-32.86)	<0.001	15.97 (5.78-44.16)	<0.001
Anaplastic any grade 3	4.88 (2.44-7.32)	5.00 (2.37-10.55)	<0.001	6.22 (2.44-15.85)	<0.001
Low-grade astrocytoma/oligodendrogliomas/mixed oligoastrocytoma* grade 2	Not reached	1.00	—	1.00	—
Extent of resection	—	1.71 (0.75-3.88)	0.20	0.35 (0.15-0.81)	0.014

NOTE: Among anaplastic mixed oligoastrocytoma ($n = 20$), rate ratio (95% CI) for AP-2α negative compared with AP-2α positive is 1.43 (0.38-5.35; $P = 0.60$).

*Reference group (i.e., category that others are compared with; rate ratio > 1, faster rate of death; rate ratio < 1, slower rate of death; rate ratio = 1, same rate of death as reference).

with normal brain by immunohistochemistry. The up-regulation of VEGF and/or MMP-2 does not always absolutely occur with AP-2α loss, which would be expected given the fact that AP-2α is only one of several regulatory genes for MMP-2 and VEGF (20). To further elaborate on the role of AP-2α in gliomagenesis, we transfected AP-2α into the U-87 glioma cell line, but this induced apoptosis (data not shown). This would be consistent with AP-2α having a proposed role as a tumor suppressor gene.

The loss of the AP-2α transcriptional factor may represent a molecular change that can be characteristic of astrocytic tumors, analogous to the allelic loss of 1p and 19q in oligodendroglioma (21, 22). In the mixed oligoastrocytoma tumors, the loss of AP-2α may be reflective of the tumor being more "astrocytic." The histopathologic definition of mixed oligoastrocytoma remains controversial (23); therefore, a reliable molecular marker may be useful in providing additional diagnostic criteria. Our data support the use of AP-2α expression to separate low-grade astrocytomas from higher-grade pure astrocytomas. Furthermore, the loss of AP-2α function may play a role in the transformation of human gliomas.

Despite the potential role of AP-2α in gliomagenesis, the prognostic effect of AP-2α remains unclear. This lack of prognostic importance may be due in part to the close association of the AP-2α loss and histologic grade or the effect of additional factors that have not yet been identified. The lack of prognostic effect is not surprising because other tumor markers have failed to show prognostic significance as well. For example, the role of the amplified *EGFR* (wild-type) and the variant (EGFRvIII) in malignant progression and effect on progression-free survival and overall survival has been debated in the literature. The amplified wild-type *EGFR* was not found to be an independent prognostic indicator of survival in several studies (24–26), and one study was inconclusive (27). One study did identify *EGFR* as a negative prognostic indicator in younger patients (28), and several others found *EGFR* to be an independent unfavorable predictor of survival (12, 29, 30). The presence of the mutated *EGFR*, EGFRvIII, was found to be an independent and significant unfavorable prognosticator of survival (31). Furthermore, p53 immunostaining showed no prognostic significance (27). The only marker that has shown prognostic significance is cathepsin B (32). Cumulatively, these data would suggest that the presence of a sole oncogene or loss of a single tumor suppressor gene does not confer prognostic effect; however, gene clusters now being elucidated with tumor microarrays may ultimately be able to provide prognostic guidance to treating oncologists and their patients.

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REFERENCES

1. Gaynor RB, Muchardt C, Xia YR, et al. Localization of the gene for the DNA-binding protein AP-2 to human chromosome 6p22.3-pter. Genomics 1991;10:1100–2.

2. Williams T, Tjian R. Analysis of the DNA-binding and activation properties of the human transcription factor AP-2. Genes Dev 1991;5:670–82.

3. Gravel M, Gao E, Hervouet-Zeiber C, Parsons V, Braun PE. Transcriptional regulation of 2′,3′-cyclic nucleotide 3′-phosphodiesterase gene expression by cyclic AMP in C6 cells. J Neurochem 2000;75:1940–50.

4. Schorle H, Meier P, Buchert M, Jaenisch R, Mitchell PJ. Transcription factor AP-2 essential for cranial closure and craniofacial development. Nature 1996;381:235–8.

5. Bar-Eli M. Role of AP-2 in tumor growth and metastasis of human melanoma. Cancer Metastasis Rev 1999;18:377–85.

6. Ruiz M, Troncoso P, Bruns C, Bar-Eli M. Activator protein 2α transcription factor expression is associated with luminal differentiation and is lost in prostate cancer. Clin Cancer Res 2001;7:4086–95.

7. Bosher JM, Williams T, Hurst HC. The developmentally regulated transcription factor AP-2 is involved in c-erbB-2 overexpression in human mammary carcinoma. Proc Natl Acad Sci U S A 1995;92:744–7.

8. Reavey-Cantwell JF, Haroun RI, Zahurak M, et al. The prognostic value of tumor markers in patients with glioblastoma multiforme: analysis of 32 patients and review of the literature. J Neurooncol 2001;55:195–204.

9. Shiraishi S, Tada K, Nakamura H, et al. Influence of p53 mutations on prognosis of patients with glioblastoma. Cancer 2002;95:249–57.

10. Pollack IF, Finkelstein SD, Woods J, et al. Expression of p53 and prognosis in children with malignant gliomas. N Engl J Med 2002;346:420–7.

11. Burton EC, Lamborn KR, Forsyth P, et al. Aberrant p53, mdm2, and proliferation differ in glioblastomas from long-term compared with typical survivors. Clin Cancer Res 2002;8:180–7.

12. Smith JS, Tachibana I, Passe SM, et al. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. J Natl Cancer Inst 2001;93:1246–56.

13. Jean D, Gershenwald J, Huang S, et al. Loss of AP-2 results in up-regulation of MCAM/MUC8 and an increase in tumor growth and metastasis of human melanoma cells. J Biol Chem 1998;273:16501–8.

14. Huang S, Jean D, Luca M, Tainsky M, Bar-Eli M. Loss of AP-2 results in downregulation of c-KIT and enhancement of melanoma tumorigenicity and metastasis. EMBO J 1998;17:4358–69.

15. Kleihues P, Ohgaki H. Primary and secondary glioblastomas: from concept to clinical diagnosis. Neuro-oncol 1999;1:44–51.

16. Ransom DT, Ritland SR, Moertel CA, et al. Correlation of cytogenetic analysis and loss of heterozygosity studies in human diffuse astrocytomas and mixed oligo-astrocytomas. Genes Chromosomes Cancer 1992;5:357–74.

17. Liang BC, Ross DA, Greenberg HS, Meltzer PS, Trent JM. Evidence of allelic imbalance of chromosome 6 in human astrocytomas. Neurology 1994;44:533–6.

18. Gershenwald J, Sumner W, Calderone T, Wang Z, Huang S, Bar-Eli M. Dominant-negative transcription factor Ap-2 augments SB-2 melanoma tumor growth *in vivo*. Oncogene 2001;26:3363–75.

19. Ruiz M, Pettaway C, Song R, Stoeltzing O, Ellis L, Bar-Eli M. Activator protein 2a inhibits tumorigenicity and represses vascular endothelial growth factor transcription in prostate cancer cells. Cancer Res 2004;64:631–8.

20. Bar-Eli M. Gene regulation in melanoma progression by the AP-2 transcription factor. Pigment Cell Res 2001;14:78–85.

21. Cairncross JG, Ueki K, Zlatescu MC, et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 1998;90:1473–9.

22. Bello MJ, Leone PE, Vaquero J, et al. Allelic loss at 1p and 19q frequently occurs in association and may represent early oncogenic events in oligodendroglial tumors. Int J Cancer 1995;64:207–10.

23. Giannini C, Scheithauer BW, Weaver AL, et al. Oligodendrogliomas: reproducibility and prognostic value of histologic diagnosis and grading. J Neuropathol Exp Neurol 2001;60:248–62.

24. Galanis E, Buckner J, Kimmel D, et al. Gene amplification as a prognostic factor in primary and secondary high-grade malignant gliomas. Int J Oncol 1998;13:717–24.

25. Newcomb EW, Cohen H, Lee SR, et al. Survival of patients with glioblastoma multiforme is not influenced by altered expression of p16, p53, EGFR, MDM2 or Bcl-2 genes. Brain Pathol 1998;8:655–67.

26. Waha A, Baumann A, Wolf HK, et al. Lack of prognostic relevance of alterations in the epidermal growth factor receptor-transforming growth factor-α pathway in human astrocytic gliomas. J Neurosurg 1996;85:634–41.

27. Bouvier-Labit C, Chinot O, Ochi C, Gambarelli D, Dufour H, Figarella-Branger D. Prognostic significance of Ki67, p53 and epidermal growth factor receptor immunostaining in human glioblastomas. Neuropathol Appl Neurobiol 1998;24:381–8.

28. Simmons ML, Lamborn KR, Takahashi M, et al. Analysis of complex relationships between age, p53, epidermal growth factor receptor, and survival in glioblastoma patients. Cancer Res 2001;61:1122–8.

29. Zhu A, Shaeffer J, Leslie S, Kolm P, El-Mahdi AM. Epidermal growth factor receptor: an independent predictor of survival in astrocytic tumors given definitive irradiation. Int J Radiat Oncol Biol Phys 1996;34:809–15.

30. Etienne MC, Formento JL, Lebrun-Frenay C, et al. Epidermal growth factor receptor and labeling index are independent prognostic factors in glial tumor outcome. Clin Cancer Res 1998;4:2383–90.

31. Shinojima N, Tada K, Shiraishi S, et al. Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme. Cancer Res 2003;63:6962–70.

32. Strojnik T, Kos J, Zidanik B, et al. Cathepsin B immunohistochemical staining in tumor and endothelial cells is a new prognostic factor for survival in patients with brain tumors. Clin Cancer Res 1999;5:559–67.

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