Prognostic Parameters in Astrocytoma: p53 and c-erbB-2 Immunoreactivity, PCNA, and AgNOR Count

Astrositomlarda Prognostik Parametreler: p53 ve c-erbB-2 İmmunoreaktivitesi, PCNA, AgNOR Sayısı

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Abstract: Most adult primary brain tumors are gliomas. These tumors are progressive, tend to recur after treatment, and are usually fatal. Some studies on the behavior of astrocytomas with similar histological features have looked at overexpression of p53 and c-erbB-2 and the immunoreactivity associated with this, at proliferative indices such as PCNA, and at AgNOR count. In this study, we identified a positive relationship between tumor progression and p53 and c-erbB-2 immunoreactivity. PCNA staining and AgNOR counts were higher in cases of tumor recurrence.

Key Words: Astrocytomas, p53, c-erbB-2, PCNA, AgNOR

Özet: Yetişkin beyin tümörlerinin çoğunluğu gliomlardır. Gliomlar progressif seyrederler, tedavi sonrası nüks etmeğe meyillidir ve fatal sonlanırlar. Benzer histolojik özellikleri taşıyan astrositom olgularında p53, c-erbB-2 expresyonları ve PCNA, AgNOR gibi proliferasyonu belirleyen bazı parametreleri inceleyen çalışmalar vardır. Bu çalışmada, tümörün prograsyonu ile p53 ve c-erbB-2 immunareaktirvitesi arasında ilişki saptadık. Pakürrens gösteren olgularda PCNA ve AgNOR sayısı yüksekti.

Anahtar Kelimeler: Astrositoma, p53, c-erbB-2, PCNA, AgNOR

INTRODUCTION

Tumors of the central nervous system account for approximately 5% of all human cancers. Sixty percent of all adult primary brain tumors are gliomas, and, of these, astrocytomas are the most common (1). These tumors are progressive, tend to recur after treatment, and are usually fatal. The progression in histological grade of astrocytic tumors from low-grade astrocytomas through anaplastic astrocytoma (AA) to glioblastoma multiforme (GM) is reflected clinically by increasingly shorter survival time (2).

Glioblastomas are a heterogeneous group of tumors that arise through multiple genetic pathways. Glioblastoma multiforme (World Health Organization [WHO] Grade IV) tumors are believed to arise either de novo or by progression from low-grade astrocytomas. Investigation has shown that there is a high frequency of p53 alteration in low-grade astrocytomas, and that p53 alterations may play a significant role in the initiation of astrocytic tumor formation (3).

The prognosis for patients with GM is dismal. The median survival time after diagnosis is approximately 50 weeks, and few patients live more than 5 years. Long-term survival is possible in approximately 5% of patients. Aggressive treatment initially and at recurrence, including maximal tumor resection and adjuvant therapy, seems to be essential for longer survival. Also, younger age and higher Karnofsky scores are associated with long-term survival in these cases (4).

The clinical behavior of a brain tumor depends on a number of factors, including host defenses, tumor location, the mass' intrinsic capacity to proliferate and infiltrate, and response to therapy. Histologic grading systems attempt to predict tumor behavior based on microscopic appearance. Grading systems for astrocytomas differ in the relative importance that they attribute to individual histological criteria, but most evaluate histological atypia, cell density, mitotic activity, microvascular proliferation, and necrosis. However, in some cases, histological grade does not reflect the behavior of the tumor. The value of the grading process is limited by many factors, including errors that occur due to observer variability and subjectivity, inadequate sampling of heterogeneous tumors, and the inability of routine histological stains to reflect important biological parameters at the chromosomal and molecular levels (5).

Some studies have found an association between ploidy and survival (5), whereas others have found no such association (6). Age at diagnosis is a strong prognostic factor for astrocytoma(4). However, other investigations have detected no association between gender and survival (7,8).

With regard to treatment, the role of radiation therapy in prolonging the survival of patients with astrocytomas is well established. Also, the extent of surgery has independent prognostic significance. Patients that undergo small biopsies have shorter survival times than those who undergo debulking or tumor resection (4).

The initiation and progression of tumors are the result of sequential or multiple genetic alterations. Two classes of genes have been shown to be target of these mutations: tumor supressor genes and oncogenes. Cellular proto-oncogens play important roles in cellular growth, differentiation, and gene regulation in non-neoplastic cells and they can be activated to dominantly transforming genes by diverse mechanisms, such as point mutation, amplification, and deregulation of expression (9,10). The p53 tumor suppressor gene is commonly mutated in human cancer. Detection of p53 abnormalities may have diagnostic, prognostic, and therapeutic implications (11). Although several proto-oncogenes are amplified in many different tumors, their role in the development of a specific human malignancy is unclear. A new human proto-oncogene similar in structure to the epidermal growth factor receptor (EGFR) has been reported and this called Her-2/neu or c-erbB-2. The function of the c-erb-2 protein in normal growth and differentiation of tumor cells remains unclear. The homology to EGFR seen in the amino acid structure of the c-erbB-2 oncoprotein would suggest that the c-erbB-2 receptor may behave similarly to EGFR.Thus, like the EGFR, it may be a potent growth stimulator (9).

AgNORs are loops of DNA that are believed to be associated with ribosomal RNA activity, protein synthesis, and cell proliferation. Studies on the behavior of astrocytomas that have similar histological features have investigated overexpression of p53 and c-erbB-2, and immunoreactivity associated with each of these, proliferative indices such as proliferating cell nuclear antigen (PCNA), argyrophilic nucleolar organizer regions (AgNORs), and DNA content (9-18). The purpose of our study was to investigate how these findings correlate with histologic grade of astrocytoma, survival time, and tumor recurrence.

MATERIALS AND METHODS

Tissue specimens were obtained from 50 astrocytoma cases, including 27 men and 23 women. The median patient age was 44 years (range, 11-64 years) for men, and 41 years (range 10-65 years) for women. The neoplasm was located in the left cerebral hemisphere in 17 patients, and in the right in 28 individuals. Of the 18 located on the left, 1 was frontal, 2 were temporal, 4 were parietal, 1 was occipital, 4 were temporo-parietal, 2 were fronto-temporal, 2 were fronto-parietal, and 1 was fronto-temporo-parietal. Of those in the right hemisphere, 6 were frontal, 1 was temporal, 12 were parietal, 4 were fronto-parietal, 2 were temporo-parietal, and 1 was parieto-occipital and 1 was parieto-temporal. Three tumors were located in the cerebellum, and 2 were in the cervical section of the spinal cord.

The astrocytomas were classified according to

the Daumas-Duport system (16) and WHO guidelines (17). The Mann Whitney Confidence Interval and Test were used to assess the association between survival and several potential prognostic factors tested that were individually. In patients with astrocytomas, ten variables were used for analysis: (1) age (years), (2) gender (male/female), (3) grade (I,II,III,IV), three location variables - (4) frontal (if the primary lesion site was mainly frontal), (5) temporal (if the primary lesion site was mainly temporal), (6) parietal (if the primary lesion was mainly parietal), (7) p53 immunoreactivity (Figure1), (8) c-erbB-2 immunoreactivity, (9) PCNA immunoreactivity (Figure 2), and (10) AgNOR count (Figure 3) .

Immunohistochemical studies were done using the streptavidin-biotin-peroxidase method. PC-l0 was used at a dilution of 1:50 to detect PCNA in paraffin sections. c-erb-2 and p53 were used at

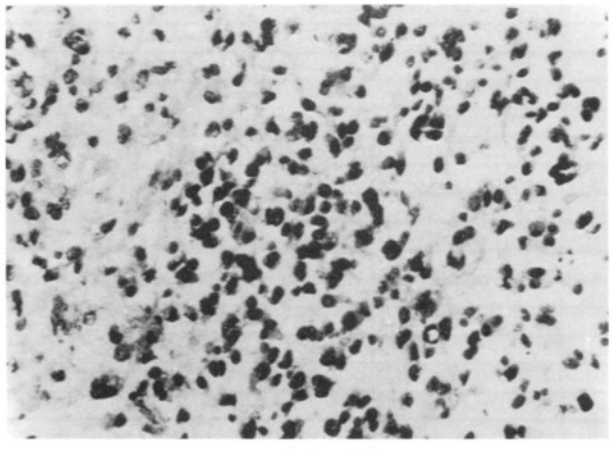

Figure 1: p53 immunoreactivity in a Grade III astrocytoma (x100).

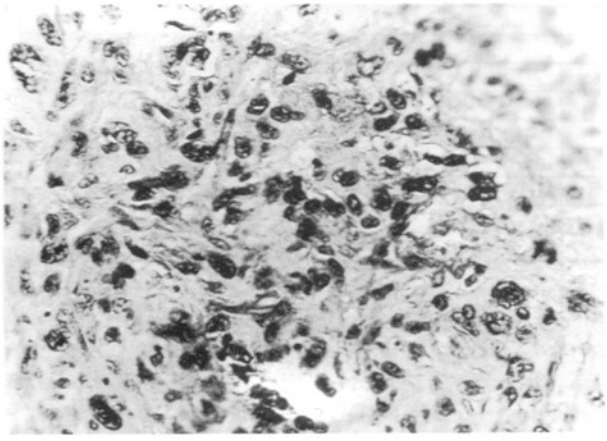

Figure 2: PCNA immunoreactivity in a Grade II astrocytoma (x2.000).

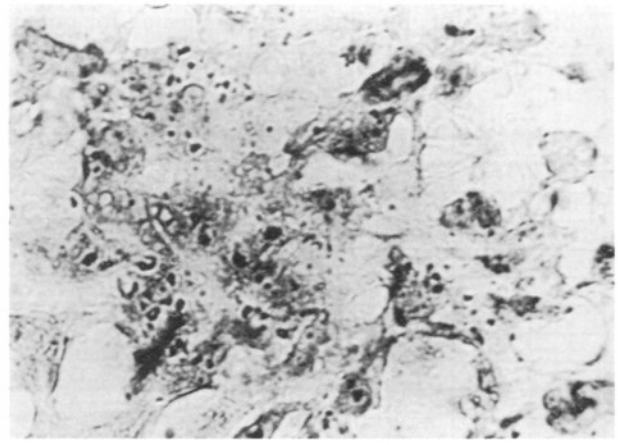

Figure 3: AgNORs in Grade III astrocytoma (oil immersion x1000)

dilutions of 1:100 and 1:50, respectively. All three monoclonal antibodies were obtained from Dakopatts (Dako., California). The colloid silver nitrate technique was used to demonstrate AgNORs, as described by Ploton et al. (18). In evaluating p53 and c-erbB-2 antibodies, tumors were classified as negative if there was no staining. Immunoreactive cases were graded as (+) if less than 10% of the tumor cells stained, (++) if 10-50% of the tumor cells stained, and (+++) if more than 50% of the tumor cells stained. The tumor cells that were immunoreactive to PC-10 were counted in ten high-power field, and cases were graded as low, moderate, or high PC-10 reactive, if there were less than 500 stained cells, from 500-1,000 positive cells, and more than 1,000 positive cells, respectively. AgNOR enumeration involved counting the nuclear dots from 1000 randomly selected neoplastic cells using a 100 X oil immersion objective.

RESULTS

Results for all of the parameters tested for each case are shown in Table I. The diagnoses were GM (28 cases), anaplastic astrocytoma (14), and differentiated astrocytoma (8). None of the patients with differentiated astrocytoma died of their disease. There were higher levels of PCNA expression, c-erbB-2 expression, and higher AgNOR counts in patients who died than in those who are currently living and in follow-up ($p<0.05$). Of the 10 variables tested, tumor grade was the one most strongly associated with survival ($p<0.05$). Age and gender were also significantly associated with survival when considered individually ($p<0.01$), but we found no association between survival and any of the three

Table I: Results for all parameters tested for patients who died of their disease or during follow-up

Case No	Age	Gender	Localization	WHO	Daumas-Duport	AgNOR count	PCNA immunoreactivity	p53 immunoreactivity	c-erbB-2 immunoreactivity	Died of disease (mounts)
1	54	M	LP	IV	GM	400	+++	-	-	16
2	50	M	RFP	IV	GM	100	-	-	-	3
3	46	M	RP	IV	GM	90	-	-	++	4
4	64	M	LTP	III	AA	80	-	-	++	1
5	42	M	RP	IV	GM	90	-	+	+++	postop
6	40	M	RP	III	AA	150	+	-	++	18
7	63	F	LFP	IV	GM	200	++	-	+++	6
8	60	M	RP	IV	GM	130	+	-	-	1
9	56	F	LO	IV	GM	350	+++	-	++	2
10	53	M	RFP	III	AA	300	+	-	++	2
11	64	M	LTP	IV	GM	280	+	-	+	4
12	63	M	LP	III	AA	100	-	-	++	1
13	41	F	LP	IV	GM	100	-	-	+	postop
14	44	F	RF	III	AA	90	+	+	+++	postop
15	32	F	RF	IV	GM	90	-	-	++	9
16	58	M	RTF	IV	GM	90	+	+	+++	9
17	34	F	RPT	IV	GM	90	-	-	++	2
18	37	F	LF	III	AA	100	++	+++	+	14
19	50	F	RP	IV	GM	90	-	+++	+++	9
20*	43	F	RP	IV	GM	300	+++	+	+++	9
21	14	F	RP	IV	GM	280	-	-	+	3
22*	26	F	LFT	IV	GM	325	+++	-	+++	8
23	50	M	RTP	IV	GM	100	-	-	+	8
24	60	M	LFTP	IV	GM	100	-	+	+++	12
25	52	M	LTP	IV	GM	100	-	-	-	14
26	51	F	RP	IV	GM	110	-	-	-	10
27*	59	M	LTP	IV	GM	250	+++	+++	+	4
28	50	M	LFP	III	AA	100	-	-	-	3
29*	42	M	RTP	IV	GM	90	-	-	+	6
30*	34	M	LP	IV	GM	90	+	-	-	16
31	65	F	RP	IV	GM	100	+	-	+++	+
32	20	F	C	PA	DA	90	-	-	-	+
33*	10	F	C	I	DA	130	-	-	-	+
34	32	M	RP	II	AA	150	-	-	++	+
35	40	F	MS	PA	DA	90	-	-	+	+
36	58	F	LFT	III	AA	95	-	-	-	+
37	11	M	MS	II	DA	87	-	-	-	+
38	19	M	C	PA	DA	85	-	-	-	+
39*	25	M	RT	IV	GM	90	+	-	-	+
40*	32	M	RP	III	AA	80	-	+	+++	+
41	36	M	RFP	III	AA	84	-	-	-	+
42	33	M	RF	II	DA	86	-	-	-	+
43	65	F	RF	IV	GM	80	-	++	++	+
44	16	M	RP	IV	GM	80	-	-	+	+
45	47	F	RFP	II	DA	80	-	-	+	+
46	34	F	RF	III	AA	90	-	-	-	+
47	56	F	LT	III	AA	80	-	-	-	+
48	38	F	RPO	IV	GM	90	+	-	+	+
49	13	F	LT	I	AA	90	-	-	-	+
50	33	M	RF	II	DA	80	-	-	-	+

* : with recurrence
F : Female
M : Male
RP : Right parietal
RF : Right frontal
RT : Right temporal
RFP : Right fronto-parietal
RPO: Right parieto-occipital
RPT : Right parieto-temporal
LT : Left temporal
LP : Left parietal
C : Cerebellum
LTP : Left temporo-parietal
LFT : Left fronto-temporal
MS : Spinal cord
PA : Pilocytic Astrocytoma
DA : Differentiated Astrocytoma
AA : Anaplastic Astrocytoma
GM : Glioblastoma multiforme
GA : Gemistocytic astrocytoma

tumor location variables (p>0.05).

Our results showed that males, older individuals, and those with higher-grade tumors, temporal or parietal lesions, or c-erbB-2-positive lesions had significantly shorter survival times. There were higher levels of AgNOR and PCNA immunoreactivity in cases with tumor recurrence.

DISCUSSION

In addition to the growth behavior of gliomas, prognosis is influenced by many other factors, including patient age, tumor location, the extent of surgical removal, p53 mutations, c-erbB-2 expression, and proliferation index.

Lang et al. (3) described the molecular genetic classification of astrocytic tumors in terms of three pathways that led to GM, namely, the progression pathway, the de novo pathway, and the alternate pathway. In the progression pathway, tumor variants are associated with p53 alterations, whereby inactivation of p53 alters normal p53 function, deregulates cell growth, and increases genetic instability. In the de novo and alternate pathways, glioblastomas arise in the absence of p53 alterations. Most of the clinical de novo glioblastomas are believed to be characterized either by loss of chromosome (Ch) 10 alone, amplification of the EGFR gene alone, loss of Ch 10 and amplification of the EGFR gene, or by loss of Ch 17p in combination with loss of Ch 9p or Ch 10 or EGFR gene amplification. Loss of putative tumor suppressor genes on different chromosomes and genes not yet identified may be responsible for the formation of the third variant of astrocytic tumors (3).

Campomenosi et al. (19) showed that chromosomes Ch 10 and Ch 17 aneuploidy was a common early event in astrocytoma development (90% of low-grade tumors were aneuploid). They found that p53 mutations and Ch 17 aneuploidy were early events, but that their incidence was not dependent on tumor grade. Loss of Ch 10 was the only alteration that was significantly correlated with tumor progression, being strongly associated with high-grade tumors. Loss of genetic material on Ch 10 and Ch 17 and gain of material on Ch 7, in association with amplification of the EGFR gene (located on Ch 7), are important factors in astrocytoma development. Amplification and rearrangement of the EGFR gene has been reported in astrocytic tumors. Amplification of the EGFR gene is known to be a late event in tumor progression (19).

It is becoming increasingly clear that the initiation and progression of tumors result from sequential or multiple genetic alterations. Two classes of genes have been identified as the targets of these mutations: tumor suppressor genes and oncogenes (2,9-11). Cellular protooncogenes play important roles in cellular growth differentiation and gene regulation in neoplastic cells, and they can be activated to transforming genes through a range of different mechanisms, including point mutation, translocation, amplification, and deregulation of expression. The products of these genes are classified according to their function and subcellular localization as nuclear proteins, cytoplasmic and membrane-associated kinases, GTP-binding proteins, membrane receptors, or growth factors. Overexpression of a protooncogene can cause cell transformation in vitro and can play a part in the neoplastic process of human tumors (8).

The results of numerous studies suggest that amplification or overexpression of this protooncogene is of prognostic significance in human breast cancer (20). In some series, the overexpression of c-erbB-2 paralleled glioma progression, and in other series there was no correlation between c-erbB-2 overexpression and postoperative relapse-free interval or overall survival time (10). In our cases, c-erbB-2 overexpression was associated with shorter survival.

The best-known cancer-related change at the gene level is mutation of tumor suppressor gene p53 (11). These mutations have been detected in 13-15% of primary breast cancers, and result in overexpression of mutant p53, which is associated with poor prognosis (20). One study showed that accumulation of cytoplasmic p53 is associated with poor prognosis in colorectal cancers (21). Frankel et al. (2) found p53 gene mutations in 40% of gliomas. Sidransky et al. (12) reported that the histological progression of brain tumors was associated with clonal expansion of p53 mutant cells.

Cytoplasmic accumulation of p53 may occur in glioblastomas, and is thought to depend on the expression of wild-type p53 alleles (13). Chozick et al. (14) found a relationship between young age and abnormal p53 immunoreactivity, independent of tumor stage. They also found mutations causing abnormal p53 immunoreactivity in 53.3% of Grade III-IV astrocytomas. Further, they noted that p53

immunoreactivity in patients with Grade II astrocytomas seems to be significantly associated with a higher recurrence rate and shorter survival time than that seen inpatients with Grade II tumors not associated with p53 immunoreactivity. These authors also showed that a certain pattern of mutant p53 expression plays a role in the progression of low-grade astrocytomas to more aggressive tumors. Their results suggested a role for p53 inactivation in the derivation of Grade III/IV astrocytomas via malignant progression from lower-grade tumors, but not in de novo tumorigenesis.

Litofsky et al. (15) reported that p53 is not important in the oncogenesis of pediatric astrocytomas, and that oncogenesis in pediatric astrocytomas may occur by different mechanisms than those associated with similar tumors in adults. Mutations in the p53 gene may be one of the initial steps in the oncogenesis of adult GM. The absence of such a mutation in pediatric astrocytomas may explain why GM is not often seen in children.

Proliferating cell nuclear antigen is an auxiliary protein of DNA polymerase delta, and is expressed in the late G1, S, G2 and M phases of the cell cycle. Experimental data suggest that PCNA expression is mainly restricted to the S-phase. Haapasalo et al. (22) found a close relationship between PCNA counts and malignancy grade in astrocytomas. Montine et al. (23) found that patients with a proliferation index (PI)of > 3 were more likely to have shorter survival than those with proliferation index < 3%. We found a positive relationship between high PCNA count and high tumor grade, and between high PCNA count and tumor recurrence.

AgNORs are loops of DNA on the short arms of acrocentric chromosomes that are believed to be associated with ribosomal RNA activity, protein synthesis, and cell proliferation(18,24,25). Most studies have shown that high AgNOR counts roughly reflect a tumor's degree of malignancy or allow differentiation among reactive, benign, and malignant conditions, but some reports do not confirm these findings (26,27). Mourad and et al. (25) found that the AgNOR count reliably reflected the proliferative activity of cells. Our results showed that AgNOR counts were higher in PCNA-positive cases.

To summarize, PCNA staining, AgNOR counts, and p53 and c-erbB-2 immunoreactivity can yield important prognostic information about astrocytomas, however, further studies are needed to confirm these results.

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