Journal of Korean Medical Science
Vol. 11, No. 6, 517-521 Dec. 1996

Expression of Androgen Receptors in Astrocytoma

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Thirty-two cases of surgically removed astrocytoma were evaluated for the expression of androgen receptors(ARs) immunohistochemically and the relationships between androgen receptors, DNA ploidy pattern, and survival of patients were studied. The cases included 18 grade I/II astrocytomas, 4 anaplastic astrocytomas, and 10 glioblastoma multiforme(GBM). Positive AR was present in 12 out of 32 cases(38%), which consisted of 5 cases in grade I/II(28%), 3 cases in anaplastic astrocytoma(75%), and 4 cases in GBM(40%). For both low and high grade astrocytomas, sex and ploidy pattern were not correlated with expression of the androgen receptors. Androgen receptor expression did not significantly affect the survival time. This study confirms previous reports of a low incidence of androgen receptors in astrocytomas. In addition, it shows that expression of androgen receptors is not correlated with DNA ploidy pattern and survival of patients in astrocytoma.

Key Words : *Androgen receptors, Astrocytoma, Ploidy, Survival*

INTRODUCTION

Brain tumors, the majority of which are of glial origin, have special characteristics as well as features typical of solid tumors. Special characteristics include their location in the brain; their interaction with the vasculature of the central nervous system and its blood-brain barrier ; a relatively low potential for metastasis but a high degree of local infiltration and invasion ; and a poor prognosis despite combined surgery, radiation, and chemotherapy. The inability to find effective treatments for patients with anaplastic astrocytomas stems in part from our limited knowledge of the basic biology and molecular genetic changes that accompany these neoplasms.

The presence of receptors for steroid hormones in tumors that are known to be hormone-dependent, such as carcinoma of the breast (McGuire et al., 1982) or prostate (Trachtenberg and Walsh., 1982), has been shown to correlate significantly with the clinical responsiveness of the tumor to hormone treatment (Loven et al., 1981 ; Loven et al., 1990).

In breast cancer, the recent receptor studies have demonstrated that androgen receptor(AR)-positive patients have a prolonged survival and better response rates to hormonal therapy than AR-negative patients (Tuelings et al., 1980 ; Bryan et al., 1984 ; Miller et al., 1985 ; Persign et al., 1985 ; Brentani et al., 1986).

Thus, it is conceivable that knowledge of the quantitive values of steroid receptor protein may more accurately detect those persons most likely to respond to endocrine therapy. Recently, following the cloning of the AR (Chang et al., 1988 ; Lubahn et al., 1988), monoclonal and polyclonal antiandrogen receptor antibodies have been prepared (Poisson and Pertuiset., 1985 ; Tan et al., 1988). To enhance our understanding of the possible pathogenic relationship between AR and astrocytomas, we studied the AR status in astrocytomas. In

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addition, we evaluated the AR expression and DNA ploidy pattern and survival of patients.

MATERIALS AND METHODS

Materials

Thirty-two cases of astrocytoma were studied. The tumor specimens were obtained from the patients who underwent brain operation between March 1991 and April 1995. Patient age ranged from 8 to 66 years (mean=39). Fifteen patients were male and seventeen female. The histological examination of surgical specimens was assessed on paraffin sections stained by hematoxylin-eosin. Tumors were classified and graded according to WHO classification.

Immunostaining procedure

All cases were studied with formalin-fixed, paraffin-embedded tissues. Five μm sections were cut on poly-L-lysine coated slides. The slides were dewaxed, rehydrated and stained using the avidin-biotin complex method. All procedures were carried out at 40℃. Endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide in absolute methanol. Normal Goat serum was used as a blocking reagent. The monoclonal antibodies used were specific for human androgen receptor (Monoclonal Anti-Androgen Receptor Antibody(IgG), Affinity Bioreagent, diluted 1:50, Neshanic Station, NJ, U.S.A). This product does not cross-react with estrogen, progesterone, or glucocorticoid receptors. The sections were incubated with these antibodies for 2 hours, followed by biotinylated rabbit immunoglobulin anti-mouse (DAKO, Calif., U.S.A) and peroxidase-conjugated streptavidin-biotin complex (DAKO, Calif., U.S.A). Slides were washed in TBS three times for 5 minutes each time. Applied 3'-diaminobenzidine tetrahydrochloride as a chromogen and incubated until desired brown color intensity has developed. After that the slides were slightly counterstained with hamatoxylin and mounted with Consul mount (Shandon, UK). A section from a prostate hypertrophy with documented androgen overexpression was used as an external positive control (Fig. 1). Negative controls consisted of a complementary section from each tumor with substitution of nonimmune serum for the primary tumor. The staining was considered as positive when a few cells or more showed positive reaction in nuclei.

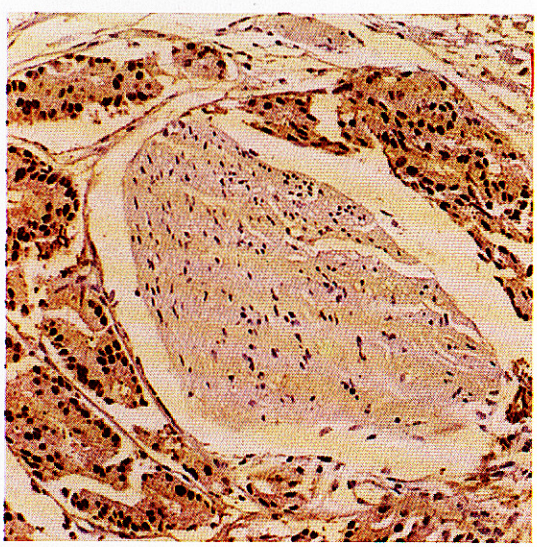

Fig. 1. On immunohistochemical stain for androgen receptor, intense nuclear reactivity for androgen receptor is seen in glandular epithelial cells of normal prostatic glands.(×200)

Statistical analysis

Statistical analysis was performed for each of the above criteria using chi-squared test or Fisher's exact test. Probability values less than 0.05 were considered to be statistically significant. The method of Kaplan and Meier was used to calculate survival and the differences between AR status was evaluated using log rank test.

RESULTS

Expressions of androgen receptors in gliomas

The immunohistochemical expression of ARs are summarized in Table 1. Among the 32 specimens examined, 12 (38%) showed positive staining for the

Table 1. Androgen receptor expression and clinicopathological variables

	Total no.	AR positivity(%)	P value
No. of specimen	32		
Sex			
Males	15	7(47)	0.52
Females	17	5(39)	
Histological type			
Astrocytoma I/II	18	5(28)	0.21
Astrocytoma III(anaplastic)	4	3(75)	
GBM	10	4(40)	
Ploidy			
Aneuploid	14	5(36)	0.85
Diploid	18	7(39)	

GBM : glioblastoma multiforme

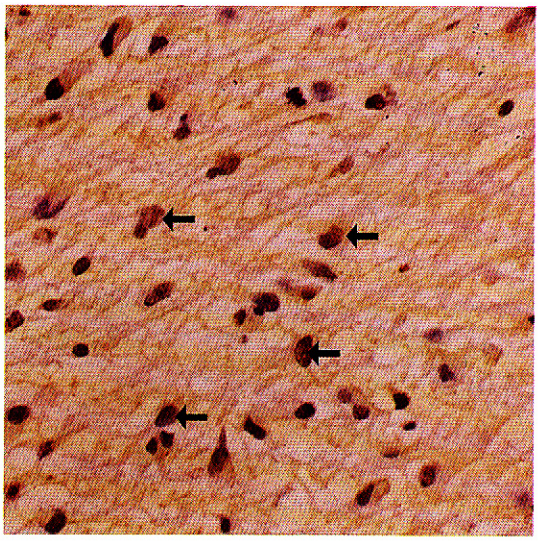

Fig. 2. Low-grade astrocytoma : The neoplastic astrocytic cells show positive nuclear reaction for androgen receptor (arrow). (×400)

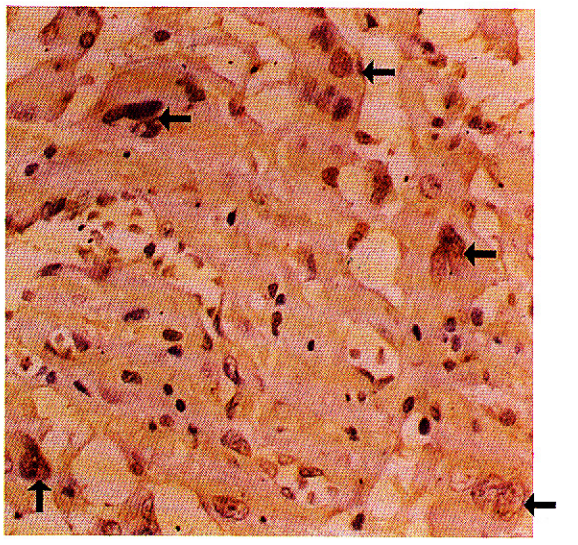

Fig. 3. Glioblastoma multiforme : Tumor cells including bizarre form show positive nuclear reaction for androgen receptor (arrow). Proliferated blood vessel do not reactive for androgen receptor. (×400)

androgen receptors in tumor cells. The positive specimens, including each histologic subtype, showed overexpression of the androgen receptors confined to nuclei of tumor cells (Figs. 2 and 3). Positive androgen receptor staining was found in 28% of the 18 patients with Grade I/II astrocytoma, 75% of the 4 patients with anaplastic astrocytoma and 40 % of the 10 patients with GBM. Comparison between AR staining patterns in histological subtypes of astrocytoma showed no significant differences (P=0.21). The intensity of immunostaining varies among tumor nuclei; some were strong, some were weak and others were negative (Figs. 2 and 3). The expression of androgen receptors were more common in males (47%), compared to 39% in female, which was not statistically significant (P=0.52).

Correlation between androgen receptor expression and flow cytometry results

The correlation between AR expression and flow cytometric results were analyzed and are summarized in Table 1. For both low (grade I/II astrocytoma) and high grade astrocytomas (anaplastic astrocytoma and GBM), ploidy pattern was not correlated with expression of the ARs (P=0.85).

Correlation between androgen receptor expression and patient survival

Figure 4 showed the survival curves of AR positive or negative groups for the patients with high and low grade astrocytomas. AR expression did not significantly affect the survival time in both groups.

DISCUSSION

Poisson and Pertuiset (1985) have reported that AR is the main steroid receptor in neuroepithelial tumors. They have recently demonstrated that 20-30% of neuroepithelial tumors are positive for AR and glucocorticoid receptor(GR). Steroid receptors present in some human solid tumors(namely breast and endocrine cancer) have been recently regarded as a possible target for therapeutic strategy both in adjuvant settings and for advanced cancer. The recent finding that steroid receptors are present in some human brain tumors (i.e, meningiomas) has therefore posed the question of whether hormonal therapies might also be useful for the clinical treatment of these tumors. Much work has been therefore done to characterize the steroid receptor (SR) pattern of the most frequent brain tumors (i.e. meningiomas and malignant neuroepithelial tumors) (Blankestein et al., 1983 ; Markwalder et al., 1984 ; Zava et al., 1984 ; Martuza et al., 1985 ; Knerich et al., 1987 ; Sica et al., 1989) and to establish their response to to in vitro hormonal manipaulation (Rolland et al., 1980 ; Markwalder et al., 1987 ; Gibelli et al., 1989). Poisson and Per-

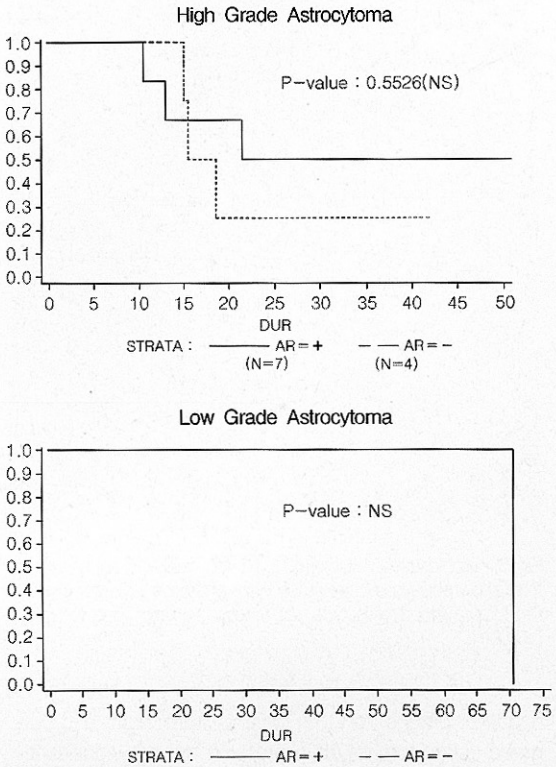

Fig. 4. Survival in patients with low grade and high grade astrocytoma according to the expression of androgen receptor.

tuiset (1985) have addressed the problem of whether SR expression in meningiomas and neuroepithelial tumors correlate with quantitative or qualitative chagnes in the synthesis of prostaglandins and thromboxane by the tumors. Some of these metabolites have in fact been shown to influence tumor proliferation which might be also affected by the presence of certain SR. They have shown that some kinetic characteristics of brain tumors can be related to different arachidonic acid(AA) metabolic patterns (Gaetani et al., 1991). There might be a relationship between the presence of selected SR, the synthesis of certain AA metabolites and proliferative patterns of brain tumors. We have considered the possibility that the difference in levels of ARs among astrocytomas was caused by the use of dexamethasone. Gliomas removed from patients treated with steroids might contained lower concentrations of androgen receptors, possibly due to the affinity of steroids to binding sites of sex hormone receptors (Jensen et al., 1976). However, since all tumor samples were analyzed following similar treatment with high-dose dexamethasone, we conclude that the expression of androgen receptor was independent of steroid treatment. We speculated that the AR expression was not correlated with proliferative potential of the tumor because the ploidy pattern was not different significantly according to the AR expression. AR expression might not be a reliable indicator for patient's survival because AR expression is not correlated with survival of patients with malignt glioma. The result of this study showing positive AR reaction in tumor cells of astrocytomas in all grades suggest the possibility of the endocrine treatment effects in these positive tumors.

ACKNOWLEDGEMENT

This work was supported by a grant from Korea University.

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