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The Effect of Isochromosome 17q Presence, Proliferative and Apoptotic Indices, Expression of c-erbB-2, bcl-2 and p53 Proteins on the Prognosis of Medulloblastoma

Medulloblastoma accounts for 20 to 25% of all intracranial neoplasms in children. The significance of the presence of isochromosome 17q (i(17q)), proliferative potential, apoptotic activity, and expression of c-erbB-2, bcl-2, and p53 proteins in predicting long-term survival of patients with medulloblastomas was investigated. Twenty children were divided into two groups (favorable and poor outcome groups). Ten children with favorable outcome (FO) were disease-free during the follow-up period (median: 61.5 months). The other ten children with poor outcome (PO) died of disease progression, having a median survival of 18 months. Fluorescent in situ hybridization (FISH) for i(17q), terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL), and immunohistochemistry for Ki-67, c-erbB-2, bcl-2, and p53 proteins was performed in these patients. Nine out of 17 children showed i(17q). There was no difference in the rate of positive i(17q) between the FO and PO groups. The presence of i(17q) was not significantly related to biological factors that we investigated. Unlike the prominent presence of the proliferative potential and p53 expression in children with PO, apoptotic activity and expression of c-erbB-2 and bcl-2 had no correlation with the outcome.

Key Words: *Medulloblastoma; Chromosomes, Human, Pair 17; Ki-67 antigen; Apoptosis; Protein p53; Prognosis*

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INTRODUCTION

Medulloblastoma (MB) accounts for 20 to 25% of all pediatric brain tumors. Unlike several other embryonal tumors, notably retinoblastoma, more study needs to be done regarding the molecular genetic events that contribute to the development and progression of MB. Clinical factors such as age, site and size of the tumor at diagnosis, extent of surgical resection, use of adjuvant chemotherapy, and radiotherapy dosage were assumed to be correlated with survival. However, the prognostic value of moleculogenetic features in MB has not yet been determined.

Although the most common cytogenetic abnormalities seen in MB are rearrangements of chromosome 17 (isochromosome 17q), the incidence is varied between 30 and 100% and the clinical significance is not determined yet (1-6). Until recently, oncogene amplification of *MYC* (c-myc), *MYCN* (N-myc), and *EGFR* (epidermal growth factor receptor), and *GLI* genes has been well documented in MB cell lines, but was found infrequently in primary biopsy specimens (7). In terms of the prognostic markers of the MB, expression of the c-erbB-2, proliferative potential, apoptotic activity have been known to be correlated with a prognosis in some analyses, but not in the others (2, 3, 8-19). The need to identify the prognostic significance of biological features of MB lead us to evaluate the factors including isochromosome 17q (i(17q)), c-erbB-2, degree of apoptosis and proliferation, bcl-2, and p53.

MATERIAL AND METHODS

Patient population

Between January 1986 and December 1995, we encountered 75 children aged less than 15 years with histologically proven posterior fossa MB at the Seoul National University Children's Hospital. Archival tissue

was available for 21 patients, forming the study population. Patients were divided into two groups: 1) favorable outcome (FO), long-term survival for more than 50 months in disease-free state, and 2) poor outcome (PO), short-term survival for less than 30 months.

Fluorescence in situ hybridization (FISH)

FISH was performed in 17 cases using kits (Oncor, Gaithersburg, MD, U.S.A.). Five μM thick sections cut from paraffin blocks were mounted onto 3-aminopropyltriethoxysilane (APS) coated slides. After deparaffinization, the sections were digested with protein-digesting enzyme (Oncor) for hybridization and denatured in 70% formamide and 2× sodium chloride/citrate buffer at 72 ℃ for 2 min. Chromosome 17 α-satellite probes (D17Z1, Oncor), which were heated to the same temperature, were added to slides and hybridized overnight at 37℃ in a humid chamber. Fluorescent isothiocyanate (FITC) and rhodamine-labeled antibodies were used for visualization. Nuclear counter-staining was performed with propidium iodine and 4'-6-diamino-2-phenylindole.

Immunohistochemical staining and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL)

Representative sections containing the largest amount of tumor, based on findings of hematoxylin and eosin staining, were selected from a single tissue block for immunohistochemical studies. Serial sections, 5 μM thick, were cut from routinely processed formalin-fixed, paraffin-embedded tissues and were mounted onto APS coated slides. After washing in phosphate buffered saline, TUNEL was performed using ApopTag In Situ Apoptosis Detection Kit (S7100-Kit, Oncor). Primary antibodies were applied and incubated as follows: Ki-67 (MIB-1, Zymed, San Francisco, CA, U.S.A., dilution 1:100); NCL-CB11 (Novacastra, Newcastle, U.K.) for c-erbB-2; monoclonal mouse antihuman bcl-2 oncoprotein clone 124 (DAKO, Carpinteria, CA, U.S.A., dilution 1:100); DO-7 antibodies for p53 protein (Novacastra).

Evaluation and statistical analysis

For identification of i(17q), centromere signals of more than 200 nuclei were assessed in each specimen under a fluorescence microscope.

Two independent pathologists (YMK and JGC) quantitatively estimated the proliferative and apoptotic indices. All sections were examined in high-power fields. The proliferative and apoptotic indices were determined by percentage of Ki-67 and TUNEL positive cells among 1,000 MB cells in 10 random regions. Immunohistochemical results for c-erbB-2, bcl-2, and p53 were recorded as positive if the tumor cells showed an unequivocally strong immunoreaction, and cases with equivocally faint staining were regarded as negative. The labelling indices were expressed in the same way as proliferative and apoptotic indices.

Independent two-group Student's t-test was used to compare the data between the two outcome groups. The correlation between biological factors was analyzed by simple linear regression. All values were expressed as mean±standard error.

RESULTS

A total of 20 patients was determined to be either FO (n=10) or PO (n=10) according to the duration of survival. One child was excluded due to operative mortality. The mean age at diagnosis in the FO and PO groups were 9.9 years and 6.6 years, respectively. At the time of the study, all patients of the FO group were alive for more than 50 months in disease-free state and the entire PO group had died within 30 months after surgery. The median follow-up period of the FO group and median survival period of the PO group were 61.5 (54- 80) and 18 (1-28) months, respectively.

Nine out of 17 tumors showed the i(17q) specific signal, i.e. two hybridization spots slightly apart from each other. The presence of i(17q) was not correlated with long-term survival, proliferative and apoptotic indices, and expression of c-erbB-2, bcl-2, and p53 proteins (Table 1).

Table 2 summarizes the correlation between biological factors and treatment outcome. Elevated Ki-67 and p53 expression was found to be related to short-term survival (p values, 0.05 and 0.03, respectively). Although the mean value of TUNEL index as well as expression of c-erbB-2 and bcl-2 was higher in the PO group than in the FO group, the difference was not statistically significant. When we exclude cases who did not undergo radiation therapy, all biological markers in this study were elevated in the PO group except for bcl-2. The elevated Ki-67 expression was significantly related with prognosis (p=0.05) and p53 overexpression had borderline significance (p=0.058).

There was a linear correlation between proliferative potential and apoptotic activity (standard coefficient= 0.68, p=0.021) and expression of p53 was related with the proliferative potential with borderline significance (standard coefficient=0.35, p=0.051). No correlations were observed between c-erbB-2 expression and proliferative potential, or bcl-2 expression and apoptotic activity.

Table 1. Clinical and biological data for 21 children with medulloblastoma

NO	Age/Sex	Surv	F/U (mos)	RT	CTx	I(17q)	Ki-67	TdT	c-erb	bcl-2	p53
1	14/F	A	80	Y	Y	-	3.4	.0	.1	.6	.5
2	9/M	A	66	Y	Y	N	4.7	.0	.0	.1	.0
3	10/F	A	65	Y	Y	Y	2.1	.1	4.1	3.7	.0
4	8/M	A	58	Y	Y	Y	.5	.3	.3	.0	.2
5	15/F	A	57	Y	Y	Y	2.3	.1	-	-	-
6	4/M	A	54	Y	Y	Y	2.5	.2	.1	.8	.1
7	6/M	A	52	Y	Y	N	1.5	.4	4.1	.0	.0
8	11/M	A	85	Y	Y	N	.7	-	-	-	-
9	15/F	A	54	Y	Y	-	1.3	-	-	-	-
10	7/M	A	66	Y	Y	N	7.6	-	.0	-	-
11	13/F	D	18	Y	Y	-	8.3	.0	3.5	1.0	1.2
12	2/M	D	19	Y	Y	-	6.5	.1	.5	.6	5.0
13	6/F	D	18	Y	N	N	1.0	.0	15.7	.2	.1
14	3/M	D	1	-	-	N	1.4	.6	.0	3.5	.7
15*	11/F	D	16	N	N	Y	.8	.0	.8	.8	.0
16	12/M	D	27	Y	Y	Y	3.9	.0	1.8	1.1	.4
17*	13/M	D	1	N	N	Y	9.0	.4	.1	.1	1.8
18	4/M	D	17	Y	Y	Y	14.8	2.6	.0	.3	.2
19	1/M	D	17	N	Y	N	3.1	1.7	3.8	5.4	3.2
20	3 mos/M	D	5	N	Y	N	8.8	.0	.3	3.1	4.2
21	4/M	D	28	Y	Y	Y	3.8	.7	1.7	.6	.1

Data of Ki-67, TdT, c-erbB-2, bcl-2, and p53 are in percents of immunopositive cells
Surv, survival status; F/U, follow-up or survival duration (months); RT, radiotherapy; CTx, chemotherapy; i(17q), isochromosome 17q; TdT, TUNEL; c-erb, c-erbB-2; A, alive; D, dead; mos, months
*These patients did not receive radiotherapy and chemotherapy because of poor general condition

Table 2. Results of statistical analysis between biological factors and treatment outcome

Factors	Group		t-test
	Favorable outcome	Poor outcome	*p* value
Age	n=10	n=10	0.13
	9.90±1.22	6.63±1.62	
Ki-67	n=10	n=10	0.05
	2.66±0.68	6.00±1.37	
TUNEL	n=7	n=10	0.21
	0.16±0.06	0.55±0.28	
c-erbB-2	n=7	n=10	0.36
	1.24±0.74	2.82±1.49	
bcl-2	n=6	n=10	0.57
	0.87±0.58	1.32±0.53	
p53	n=6	n=6	0.03
	0.13±0.08	1.62±0.59	

Data of Ki-67, TUNEL, c-erbB-2, bcl-2, and p53 are in percents of immunopositive cells
n, number of patients
Data were shown as mean±standard error

DISCUSSION

There have been many controversies regarding the association between deletion of chromosome 17p and clinical outcome (2, 3, 10). In our analysis, the presence of i(17q) made no difference in terms of long-term survival. When we exclude the radiation effect, the incidence of i(17q) was 50% (4 out of 8) in the FO group and 75% (3 out of 4) in PO group. To clarify the relationship between i(17q) and prognosis, multivariated analysis including clinical factors in the large series was needed. The presence of i(17q) in MB results in deletion of the short arm and gain of the long arm of chromosome 17 (1-6). Therefore, the loss of a tumor suppressor gene, which is located at 17p, as well as the addition of a certain gene on 17q may result in increased production of normal or abnormal proteins. Several genes were isolated from 17p13 distal to TP53 and examined for mutations. At present, there are no good candidates for this locus (10). The location of c-erbB-2 proto-oncogene is 17q11.2-12, and thus this oncogene is duplicated in tumors with an i(17q) and might be involved in the pathogenesis of MB. However, its potential role in both the pathogenesis and prognosis of MB is still controversial. Gilbertson et al. (8) demonstrated that the c-erbB-2 oncogene product is expressed in a majority of MB. In a retrospective immunohistochemical study of 55 MB, patients whose tumors contained more than 50% of cells that expressed the c-erbB-2 protein had a significantly worse prognosis than patients with less than 50% positive tumor cells. Herms et al. (9) reported that the expression of c-erbB-2 is much less common in MB

than Gilbertson et al. (8), particularly in young children (less than 3 years old). It seems worthwhile to note that the discrepancies in the reported rates of c-erbB-2 expression may be due to the sparse rim of cytoplasm in characteristic MB cells that make it difficult to interpret the data of cell membrane or cytoplasmic immunostaining. In our study, c-erbB-2 protein overexpression was neither evident nor correlative with the presence of i(17q). Furthermore, there was no clinical significance in terms of long-term survival. These findings suggest that additional loci may be involved in the initiation and progression of these tumors such as the loss of all or parts of chromosome 6 which is usually found in tumors that have more genetic changes other than i(17q). Nonetheless, the expression of c-erbB-2 protein and functional status of c-erbB-2 gene must be investigated in a larger group of children with MB.

Tumor suppressor gene TP53 was considered an excellent candidate for the 17p locus because it is the most commonly mutated tumor suppressor gene in cancers. Mutations in p53 gene may cause an accumulation of p53 protein. Jaros et al. (11) stated that intense overexpression of p53 protein can identify a group of MB patients with increased risk of death. In our series, p53 overexpression is associated with poor prognosis even when children who did not receive radiation therapy were excluded. However, MB appears to be one of the few tumors that do not contain mutations in this gene (12-14). Therefore, further study on the mechanism of p53 overexpression other than p53 mutation is needed.

The reported Ki-67 labelling index (LI) of MB ranges from 14.9 to 56.5% (16, 17), higher than in our study. Ki-67 LI showed that cell proliferation rather than apoptosis may be the key to predict long-term survival of MB.

The results of studies on the apoptotic activity vary among different tumors, and the prognostic value of apoptosis seems tumor-specific. Apoptotic activity calculated by Haslam et al. (15) was a strong indicator of treatment outcome for children with MB, showing that a high apoptotic activity was a good prognostic factor. However, our result did not show correlation between long-term survival and apoptotic activity. The apoptotic activity of MB by Schiffer et al. (16), as measured by TUNEL, ranges from 0.36 to 1.3, and thus comparable to ours. However, according to a study by Haslam et al. (15), the median tumor tissue apoptotic activity for the cohort of 43 patients is 7.5 (range 0.2 to 85.7), which is higher than our result. Our result on the correlation between proliferative and apoptotic indices shows that hyperproliferative cells might be more subject to apoptosis, resulting in higher proliferative and apoptotic indices.

Many studies on apoptosis have focused on p53 and bcl-2 expression. Bcl-2, a cellular protein that inhibits apoptosis, has been studied in a small number of patients with MB, but no correlation between bcl-2 expression and survival of children with MB has been identified as in our present study (16, 18, 19). Furthermore, there was no correlation of p53 and bcl-2 expression with apoptotic activity.

Many factors are known to be associated with the prognosis of MB such as age, differentiation, extent of surgical removal, involvement of eloquent area, drop metastasis, and treatment modalities. The prognostic values of each moleculogenetic factor in our 20 MB patients have their own significance. However, for ideal evaluation of the prognostic values, it would be better to do further analysis by adjusting these risk factors between the FO and PO groups. Because of limited number of patients and numerous prognostic parameters, we could not perform multivariate analysis. Instead, only the influence of radiation therapy was adjusted. Another analysis was also performed in patients who received radiation therapy to exclude the influence of radiation therapy.

We conclude that the presence of the i(17q) was not associated with the treatment outcome. The proliferative potential and p53 protein expression should be further investigated.

REFERENCES

1. Albrecht S, von Deimling A, Pietsch T, Giangaspero F, Brandner S, Kleihues P, Wiestler OD. *Microsatellite analysis of loss of heterozygosity on chromosomes 9q, 11p and 17p in medulloblastomas. Neuropathol Appl Neurobiol 1994; 20: 74-81.*
2. Batra SK, McLendon RE, Koo JS, Castelino-Prabhu S, Fuchs HE, Krischer JP, Friedman HS, Bigner DD, Bigner SH. *Prognostic implications of chromosome 17p deletions in human medulloblastomas. J Neurooncol 1995; 24: 39-45.*
3. Biegel JA, Janss AJ, Raffel C, Sutton L, Rorke LB, Harper JM, Phillips PC. *Prognostic significance of chromosome 17p deletions in childhood primitive neuroectodermal tumors (medulloblastomas) of the central nervous system. Clin Cancer Res 1997; 3: 473-8.*
4. Cogen PH, Daneshvar L, Metzger AK, Duyk G, Edwards MS, Sheffield VC. *Involvement of multiple chromosome 17p loci in medulloblastoma tumorigenesis. Am J Hum Genet 1992; 50: 584-9.*
5. Emadian SM, McDonald JD, Gerken SC, Fults D. *Correlation of chromosome 17p loss with clinical outcome in medulloblastoma. Clin Cancer Res 1996; 2: 1559-64.*
6. Giordana MT, Migheli A, Pavanelli E. *Isochromosome 17q is a constant finding in medulloblastoma. An interphase cytogenetic study on tissue sections. Neuropathol Appl Neurobiol*

1998; 24: 233-8.

7. Wasson JC, Saylors RL III, Zeltzer P, Friedman HS, Bigner SH, Bugner PC, Bigner DD, Look AT, Douglass EC, Brodeur GM. *Oncogene amplification in pediatric brain tumors. Cancer Res 1990; 50: 2987-90.*
8. Gilbertson RJ, Pearson AD, Perry RH, Jaros E, Kelly PJ. *Prognostic significance of the c-erbB-2 oncogene product in childhood medulloblastoma. Br J Cancer 1995; 71: 473-7.*
9. Herms JW, Behnke J, Bergmann M, Christen HJ, Kolb R, Wilkening M, Markakis E, Hanefeld F, Kretzschmar HA. *Potential prognostic value of c-erbB-2 expression in medulloblastomas in very young children. J Pediatr Hematol Oncol 1997; 19: 510-5.*
10. Cogen PH, McDonald JD. *Tumor suppressor genes and medulloblastoma. J Neurooncol 1996; 29: 103-12.*
11. Jaros E, Lunec J, Perry RH, Kelly PJ, Pearson AD. *p53 protein overexpression identifies a group of central primitive neuroectodermal tumours with poor prognosis. Br J Cancer 1993; 68: 801-7.*
12. Felix CA, Slavc I, Dunn M, Strauss EA, Phillips PC, Rorke LB, Sutton L, Bunin GR, Biegel JA. *Germline and somatic p53 gene mutations in pediatric brain tumors. Med Pediatr Oncol 1995; 25: 431-6.*
13. Raffel C, Thomas GA, Tishler DM, Lassoff S, Allen JC. *Absence of p53 mutations in childhood central nervous system primitive neuroectodermal tumors. Neurosurgery 1993; 33: 301-6.*
14. Saylors RL, Sidransky D, Friedman HS, Bigner SH, Bigner DD, Volgelstein B, Brodeur GM. *Infrequent p53 gene mutations in medulloblastomas. Cancer Res 1991; 51: 4721-3.*
15. Haslam RH, Lamborn KR, Becker LE, Israel MA. *Tumor cell apoptosis present at diagnosis may predict treatment outcome for patients with medulloblastoma. J Pediatr Hematol Oncol 1998; 20: 520-7.*
16. Schiffer D, Cavalla P, Chio A, Giordana MT, Marino S, Mauro A, Migheli A. *Tumor cell proliferation and apoptosis in medulloblastoma. Acta Neuropathol 1994; 87: 362-70.*
17. Onda K, Davis RL, Edwards MS. *Comparison of bromodeoxyuridine uptake and MIB 1 immunoreactivity in medulloblastomas determined with single and double immunohistochemical staining methods. J Neurooncol 1996; 29: 129-36.*
18. Heck K, Pyle P, Bruner JH. *Bcl-2 expression in cerebellar medulloblastomas [abstract]. Brain Pathol 1994; 4: 438.*
19. Nakasu S, Nakasu Y, Nioka H, Nakajima M, Handa J. *Bcl-2 protein expression in tumors of the central nervous system. Acta Neuropathol 1994; 88: 520-6.*