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ORIGINAL ARTICLE

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Analysis of immunohistochemical expression of p53 and the proliferation marker Ki-67 antigen in skull base chordomas: relationships between their expression and prognosis

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Abstract The prognosis of chordomas is difficult to predict based solely on histological findings. The purpose of this study was to assess the immunohistochemical expression of the proliferation marker Ki-67 antigen and the expression of p53 in skull base chordomas and to relate their expressions to the outcome. We examined the expression of p53 and the MIB-1 labeling index (LI), assessed by Ki-67 expression, in 19 tumors (initial, $n = 11$; recurrent, $n = 8$) from 11 patients. The correlation among the MIB-1 LI, p53 expression, and the clinical outcome was analyzed. The mean MIB-1 LI and p53 expression at the initial surgery were 5.6 ± 4.6% and 9.0 ± 9.4%, respectively. At the time of recurrence, the mean MIB-1 LI and p53 expression were 10.2 ± 7.4% and 16.5 ± 12.0%. The correlation between the MIB-1 LI and p53 expression at the initial and recurrent surgeries was highly significant ($r = 0.948$; $P < 0.0001$). The change in p53 expression from the initial to the recurrent chordomas was significantly greater in patients who died of tumor-related causes than in the surviving patients. In the surviving patients, the values for MIB-1 LI and p53 expression in the recurrent tumors were significantly higher in the disease-ongoing group than in the disease-free group. Our results suggest that determination of the immunohistochemical expression of p53 and Ki-67 antigen is helpful to predict tumor recurrence and prognosis in skull base chordomas.

Key words Skull base chordoma · MIB-1 · p53 · Recurrence · Prognosis

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Introduction

Chordoma is a rare tumor arising from the notochordal remnants. In an epidemiologic study in a large series of patients with chordoma in the United States,[1] it was reported that median survival was 6.29 years and that 5- and 10-year relative survival rates were 67.6% and 39.9%, respectively. Although chordomas are histologically benign, the recurrence rate is very high and the prognosis is poor.[2,3] Approximately 35% are located at the skull base, and they mostly involve the clivus. Complete resection is limited due to their location. Radiotherapy did not improve the survival time, but there was a trend toward prolonged disease-free survival.[4] Because of the tendency of chordomas to recur, an operation should offer the opportunity for repeat surgery. It is difficult to manage skull base chordomas, and it is also difficult to predict their recurrence and prognosis.

Alterations of the *p53* tumor suppressor gene play a major role in the tumorigenesis of various human malignant tumors.[5–7] The majority of the *p53* alterations are mutations, and mutant *p53* products result in the accumulation of these nuclei in tumor cells and in the detection of the p53 protein by immunohistochemistry. Few reports, however, have described either *p53* gene mutation or the expression of p53 protein in relation to chordomas or evaluated the relationship among p53 expression, proliferation markers, and clinical factors in these tumors.[8] There have been no reports investigating the expression of p53 and the proliferation marker Ki-67 antigen in specimens obtained at either initial or recurrent surgeries.

The objectives of this study were to investigate the immunohistochemical expression of p53 and Ki-67 antigen in skull base chordomas at the initial and recurrent surgeries, and to assess whether p53 expression and the MIB-1 labeling index (MIB-1 LI), assessed by Ki-67 expression, have prognostic value.

Table 1. Clinical characteristics and percent expression of MIB-1 and p53 in 11 patients

Patient no.	Age (years)/Sex	Initial tumor size (mm^3)	Td (months)	MIB-1 labeling index (%)		p53 (%)		Follow-up period (years)	Outcome (KPS; %)
				Initial op.	Reop.	Initial op.	Reop.		
1	41/F	77.5	3.2	11.3	19.0	23.1	31.9	8.3	Dead (0)
2	53/F	27.8	5.3	3.3	17.7	2.9	24.8	8.7	Dead (0)
3	60/F	161.9	6.2	1.6	6.2	6.2	17.7	9.3	Dead (0)
4	47/M	54.0	4.0	2.8	7.2	0	11.8	18.9	Dead (0)
5	53/M	52.7	7.6	5.1	–	5.5	–	4.6	Survived (90)
6	52/M	8.7	–	3.6	–	4.2	–	5.6	Survived (90)
7	71/F	6.1	5.4	10.3	–	13.9	–	7.8	Survived (60)
8	48/F	96.9	1.7	11.8	18.3	19.3	28.1	8.1	Survived (80)
9	65/F	19.5	10.0	10.8	10.6	24.3	17.8	17.6	Survived (80)
10	9/M	15.0	82.0	0.2	0.1	0	0	23.5	Survived (90)
11	38/F	19.2	37.6	0.5	2.6	0	0	28.6	Survived (70)
	Mean	49.0	16.3	5.6	10.2	9.0	16.5	12.8	

Td, tumor volume doubling time; KPS, Karnofsky performance status

Patients, materials, and methods

Patients and tissue samples

This study included 11 patients with skull base chordomas who were treated at Shinshu University Hospital between 1978 and 2005. There were five male patients and six female patients, aged 9 to 71 years (mean, 48.8 years) at the initial operation (Table 1). The mean follow-up period was 12.8 years (range, 4.6–28.6 years). Tumor resection was performed a total of 61 times (mean, 5.5 times) in the 11 patients. In 8 of the 11 patients, tumor excision was performed more than twice because of tumor recurrence. Six patients underwent fractionated radiotherapy. Gamma knife surgery was performed for 14 lesions in 7 patients. Four patients died due to tumor growth and 7 were alive. The details of the 11 patients were retrospectively examined for initial tumor size, tumor volume doubling time (Td), and clinical outcome.

Nineteen chordomas from 11 patients were examined immunohistochemically for the expression of p53 and the proliferation marker, Ki-67 antigen. In 8 patients with recurrent chordomas, tumor specimens obtained at the surgery for the recurrent tumor as well as those obtained at the initial surgery were used.

Immunohistochemistry

Paraffin-embedded sections were submitted for immunohistochemistry. Deparaffinized 4-μm-thick sections were immunostained after antigen retrieval with microwave heating in 0.01 M citrate buffer (pH 6.0). The sections were incubated with anti-p53 antibody (Transduction Laboratory, Lexington, Kentucky, USA), or the anti-Ki-67 antibody, MIB-1 (Immunotech, Marseilles, France). As the secondary antibody, Envision$^+$ (DAKO, Glostrup, Denmark), which consists of dextran polymers conjugating a large number of goat antibodies against mouse immunoglobulins and horseradish peroxidase, was used. Counterstaining was performed with hematoxylin. To calculate in a double-blind fashion, we counted 1000 or more cells, and the percent expressions of Ki-67 antigen (the MIB-1 LI) and p53 were calculated.

Statistical analysis

Statistical analysis was done with Student's *t*-test. The mean value, SD, and *P* value were calculated. A *P* value of less than 0.05 was regarded as statistically significant.

Results

Immunohistochemical findings of Ki-67 antigen and p53 (Table 1)

Ki-67 antigen and p53 were detected in the nuclei of chordoma tumor cells, confirming the validity of the immunostaining (Fig. 1 A–C). The MIB-1 LI (which represents the percentage of all tumor cells that are Ki-67-positive) was 5.6 ± 4.6% (mean) at the initial operation. At reoperation the mean MIB LI was higher than that at the initial operation, but the difference was not statistically significant (Fig. 2). The mean percent expression of p53 was 9.0 ± 9.4% at the initial operation and 16.5 ± 12.0% at the time of recurrence. The mean p53 expression at reoperation was higher than that at the initial operation, but the difference was not statistically significant (Fig. 3). The correlation between the MIB-1 LI and p53 expression at the initial operation was highly significant ($r = 0.937$; $P < 0.0001$; Fig. 4 A). The correlation between the MIB-1 LI and p53 expression at the initial and recurrent operations was also highly significant ($r = 0.948$; $P < 0.0001$; Fig. 4 B). The elevation of p53 expression from the initial to the recurrent tumors was prominent in patients with tumor-related death (patients 2, 3, and 4, as numbered in Table 1).

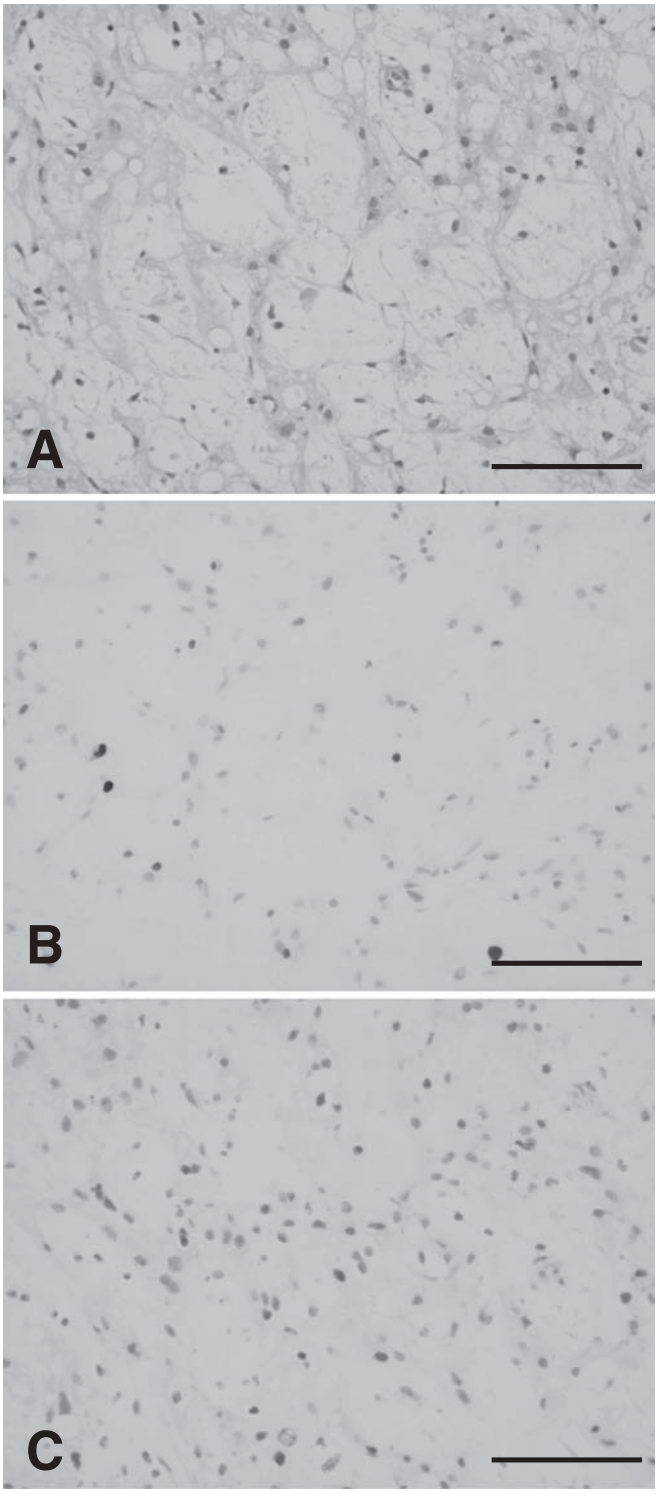

Fig. 1A–C. Photomicrographs of the surgical specimen obtained at the initial operation in patient 9 (as listed in Table 1), showing H & E staining (**A**) and immunohistochemical staining for Ki-67 antigen using MIB-1 antibody (**B**) and p53 antibody (**C**). *Bars*, 100 μm

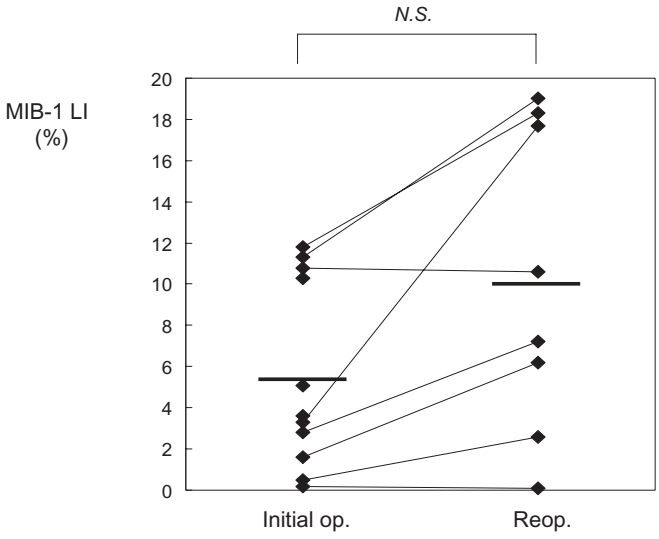

Fig. 2. The mean MIB-1 labeling index (*LI*) was 5.6 ± 4.6% at the initial surgery (*initial op.*) and 10.2 ± 7.4% at the time of recurrence (*reop.*). The former value was lower than the latter, but the difference was not significant (*N. S.*). *Horizontal bars* show mean values

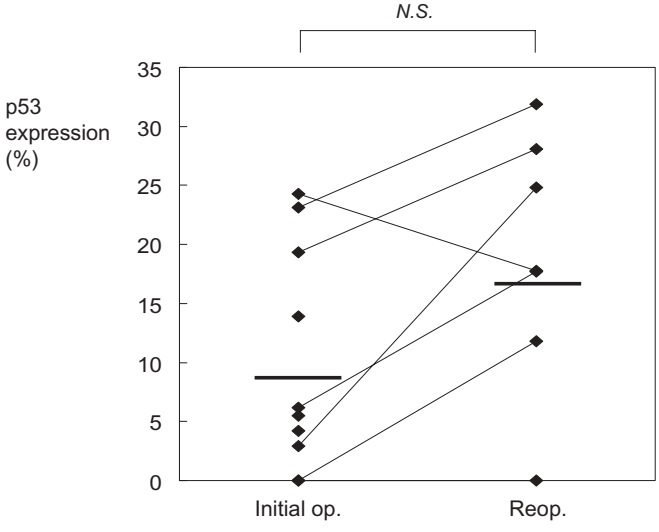

Fig. 3. The mean percent expression of p53 was 9.0 ± 9.4% at the initial surgery and 16.5 ± 12.0% at the time of recurrence. The former value was lower than the latter, but but the difference was not significant. *Horizontal bars* show mean values

Initial tumor size and tumor volume doubling time (Td; Table 1)

The mean initial tumor size was 49.0 mm^3 (range, 6.1–161.9 mm^3). The mean Td was 16.3 months (range, 1.7–82.0 months). The MIB-1 LI was not significantly associated with the initial tumor size or Td. The expression of p53 was also not significantly associated with the initial tumor size or Td.. High values for MIB-1 LI and p53 expression were associated with earlier tumor recurrence due to short Td; however, the association was not significant.

Table 2. Characteristics of survival and dead groups

Clinical characteristics	Survival group ($n = 7$)	Dead group ($n = 4$)	P value
Age (years)	48.0 ± 20.3	50.3 ± 8.1	0.8398
Initial tumor size (mm^3)	31.1 ± 32.8	80.3 ± 58.1	0.1009
Tumor volume doubling time (months)	63.3 ± 125.3	4.7 ± 1.3	0.3861
MIB-1 at initial op.	6.0 ± 4.9	4.8 ± 4.4	0.6753
MIB-1 at reop.	7.9 ± 8.3 ($n = 4$)	10.4 ± 6.4	0.4192
MIB-1	2.2 ± 3.4 ($n = 4$)	7.8 ± 4.7	0.1022
p53 at initial op.	9.6 ± 9.7	8.1 ± 10.3	0.8082
p53 at reop.	11.5 ± 13.9 ($n = 4$)	21.6 ± 8.7	0.2653
p53	0.6 ± 6.3 ($n = 4$)	14.2 ± 5.6	0.0176*

MIB-1 LI = (MIB-1 LI at reop.) – (MIB-1 LI at initial op.)
p53 = (p53 at reop.) – (p53 at initial op.)
*Significant difference

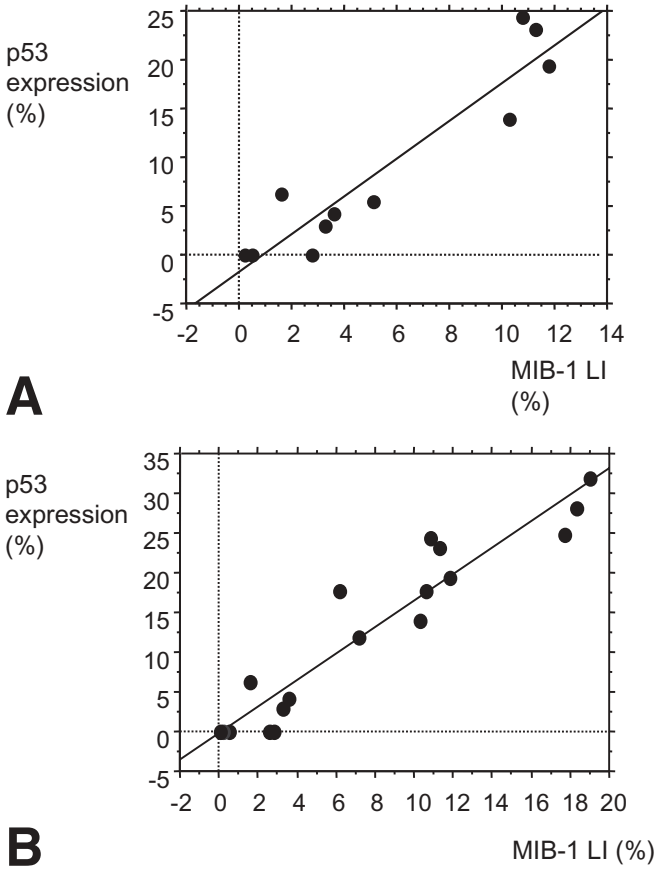

Fig. 4. A. The correlation between the MIB-1 LI and p53 expression at the initial surgery was highly significant ($r = 0.937$; $P < 0.0001$). **B** The correlation between the MIB-1 LI and p53 expression at the initial and recurrent surgeries was also highly significant ($r = 0.948$; $P < 0.0001$)

Prognostic value

Statistical analysis of characteristics such as age, initial tumor size, Td, MIB-1 LI at the initial operation and reoperation, and p53 expression at the initial operation and reoperation was performed to determine differences between patients who survived (survival group) and those who had died (dead group; Table 2). The only significant difference between the groups was in the change of p53 expression from the initial operation to reoperation.

Statistical analysis of the above characteristics was also performed to determine differences between patients with no recurrence more than 2 years after the final treatment (disease-free survival group) and patients with recurrence within the past 2 years (disease-ongoing group; including patients who had died of tumor-related causes; Table 3). The disease-free survival group consisted of patients 5, 6, 7, 10, and 11. The disease-ongoing group consisted of patients 1, 2, 3, 4, 8, and 9. The values for MIB-1 LI and p53 expression in the recurrent chordomas in the disease-ongoing group were significantly higher than those in the recurrent chordomas in the disease-free survival group. The log-rank test revealed no significant differences in survival between these two groups.

Discussion

We investigated the immunoreactivity of p53 protein and the proliferative marker Ki-67 antigen, as molecular markers, in skull base chordomas. In the current study, the correlation between the percent expression of p53 and the MIB-1 LI was highly significant for both the initial and the recurrent tumors. The examination of both p53 expression and the MIB-1 LI was important and essential for the prediction of future recurrence and prognosis in patients with skull base chordoma.

Ki-67, which is expressed at all stages of mitosis except for G0, is one of the reliable markers of cell proliferation. MIB-1 is an antibody reactive to this protein. A correlation between the MIB-1 LI and survival has been described in cancer patients. The MIB-1 LI of gliomas has been shown to correlate well with poorer survival.[9,10] Few studies, however, have reported that the MIB-1 LI was correlated with tumor recurrence and low survival rate in patients with chordoma.[11,12] Ki-67 LIs in excess of 6% were observed with faster-growing skull base chordomas.[13] In our series, the MIB-1 LI was relatively higher in the recurrent tumors than in the initial tumors. The MIB-1 LI in the recurrent tumors was higher than that in the initial tumors in the disease-ongoing group (which included patients who had died of

Table 3. Characteristics of disease-free and disease-ongoing groups

Clinical characteristics	Disease-free survival group ($n = 5$)	Disease-ongoing group ($n = 6$)	P value
Age (years)	44.6 ± 23.1	52.3 ± 8.9	0.4656
Initial tumor size (mm^3)	20.3 ± 18.8	72.9 ± 52.5	0.0636
Tumor volume doubling time (months)	92.1 ± 151.1	5.1 ± 2.8	0.1837
MIB-1 at initial op.	3.9 ± 4.1	6.5 ± 4.4	0.3501
MIB-1 at reop.	1.4 ± 1.8 ($n = 2$)	12.0 ± 5.7	0.0365*
MIB-1	1.0 ± 1.6 ($n = 2$)	6.3 ± 4.8	0.1944
p53 at initial op.	4.7 ± 5.7	12.6 ± 10.8	0.1768
p53 at reop.	0 ($n = 2$)	22.0 ± 7.5	0.0078*
p53	0 ($n = 2$)	9.9 ± 9.4	0.2076

Disease-free survival group, no recurrence more than 2 years after final treatment; disease-ongoing group, uncontrollable tumor due to recurrence within past 2 years (includes patients who died of tumor-related causes)
MIB-1 LI = (MIB-1 LI at reop.) – (MIB-1 LI at initial op.)
p53 = (p53 at reop.) – (p53 at initial op.)
*Significant difference

tumor-related causes). Our results suggest that the MIB-1 LI is associated with tumor recurrence and poor prognosis.

The tumor suppressor gene *p53* plays a role in maintaining the genetic integrity of cells. The wild-type p53 protein is involved in the negative regulation of cell proliferation, whereas the aberrant p53 protein has lost the negative regulation of cell growth. Matsuno et al.[14] reported that the immunohistochemically positive staining of p53 correlated well with a high MIB-1 LI and the recurrence of intracranial chordomas. Kilgore and Prayson,[15] however, reported that p53 immunoreactivity did not appear to reliably correlate with adverse outcome. Naka et al.[8,16] reported that the MIB-1 LI in two chordomas with p53 overexpression was significantly higher than that in eight chordomas without p53 overexpression, and p53 overexpression was associated with reduced survival. Pallini et al.[17] showed that, in p53-positive chordomas, the interval for tumor recurrence was significantly shorter than that in p53-negative chordomas. In our study, the expression of p53, and the MIB-1 LI, were relatively higher in the recurrent tumors than in the initial tumors. To our knowledge, this is the first report investigating the expression of p53 in chordoma specimens obtained at both the initial and recurrent surgeries. The elevation of p53 expression from the initial to recurrent tumors was prominent in three patients who died of tumor-related causes. The MIB-1 LI in the recurrent chordoma was also elevated in these patients. In one of the dead patients and two of the disease-ongoing patients, high expression of p53 (at more than 15%) was observed in the initial and recurrent chordomas. High expression of p53 was associated with recurrent tumor and poor prognosis. Thus, the expression of p53 and the MIB-1 LI are both associated with tumor recurrence and poor prognosis.

Although the role of p53 protein in the tumorigenesis of chordoma is unclear, tumor progression may depend on the p53 pathway. Mutation of p53 may play an important role in the formation of chordomas, especially progressive recurrent chordomas. Further studies are needed to clarify the tumor biology of skull base chordomas.

Conclusion

The correlation between p53 expression and the MIB-1 LI was highly significant in skull base chordomas. Both the MIB-1 LI and p53 expression are helpful to predict tumor recurrence and prognosis. In particular, p53 expression could be an important marker for predicting outcome at the time of recurrence. Therefore, we recommend the routine assessment of p53 expression and the MIB-1 LI in skull base chordomas.

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