Table 2 Surgical outcomes after posterior decompression with instrumented fusion

Surgical outcomes	Before surgery	After surgery				
		3 months	6 months	9 months	1 year	Final FU
JOA score (points)[a]	3.7 ± 1.4 (1.0–6.5)	6.4 ± 1.8[†] (3.5–11.0)	7.3 ± 2.0[†] (4.0–11.0)	7.7 ± 2.0[†,‡] (4.0–11.0)	7.9 ± 2.0[†,‡] (4.0–11.0)	8.0 ± 2.0[†,‡] (4.0–11.0)
Recovery rate (%)[a,b]		36.7 ± 23.4 (0–100)	48.8 ± 26.5 (14.3–100)	54.0 ± 26.9[§] (14.3–100)	56.8 ± 27.4[§] (14.3–100)	58.1 ± 27.5[§] (14.3–100)

FU follow-up, *JOA* Japanese Orthopedic Association (full score = 11 points)

[*] Statistically different from the JOA score before surgery ($p < 0.05$)

[†] Statistically different from the JOA score at 3 months after surgery ($p < 0.05$)

[§] Statistically different from the recovery rate at 3 months after surgery ($p < 0.05$)

[a] Values are expressed as the mean ± standard deviation, with the range in parenthesis

[b] Recovery rate = $\frac{\text{Postop JOA score} - \text{preop JOA score}}{\text{Full score (11)} - \text{preop JOA score}} \times 100$

(10% ≤ recovery rate < 50%), unchanged (0% ≤ recovery rate < 10%), or worsened (recovery rate < 0%).

Radiographic assessment

In our last six consecutive patients (Case 19 through Case 24), we measured preoperatively the kyphotic angles at the instrumented fusion levels in the supine, prone, and sitting positions. To calculate the kyphotic angle, we measured the sagittal Cobb angle between the upper endplate of the uppermost vertebra and the lower endplate of the lowest vertebra at the instrumented fusion levels [ ]. To accurately identify the landmarks for the angle measurement, we controlled the contrast and brightness of the digital images of the thoracic spine. The kyphotic angle was measured by three spine surgeons independently, and we defined the mean value of these measurements as the value of the kyphotic angle.

Intraoperative spinal ultrasonography

After laminectomy, we assessed the posterior shift of the spinal cord away from the anterior ossified mass by means of intraoperative ultrasonography. We looked for the presence of the subarachnoid space on the ventral side of the spinal cord, on the basis of which we classified the decompression status as either non-contact type or contact type [,]. The non-contact type is characterized by visualization of the subarachnoid space between the OPLL and the spinal cord, indicating that sufficient decompression of the spinal cord from the anterior ossified mass has been achieved. Conversely, in the contact type, the spinal cord always touches the OPLL, and no subarachnoid space is evident between the OPLL and the spinal cord, indicating persistent impingement of the spinal cord from the anterior direction even after the posterior decompression procedure.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U* test. A p value <0.05 was considered statistically significant. Results are presented as the mean ± standard deviation of the mean.

Results

Surgical outcome

The mean JOA score before surgery was 3.7 (range 1.0–6.5). All patients showed neurological recovery at the final follow-up; the mean JOA score at final follow-up was 8.0

(range 4.0–11.0 points), and the mean recovery rate was 58.1% (range 14.3–100%) (Table 2).

The mean JOA score was 6.4 at 3 months after surgery, 7.3 at 6 months after surgery, 7.7 at 9 months after surgery and 7.9 at 12 months after surgery (Table 2). The JOA scores at 3 months after surgery and later were significantly higher than the JOA score before surgery. In addition, the JOA scores at 9 months after surgery and later were significantly higher than the JOA score at 3 months after surgery (Table 2). The mean recovery rate was 36.7% at 3 months after surgery, 48.8% at 6 months after surgery, 54.0% at 9 months after surgery and 56.8% at 12 months after surgery (Table 2). The recovery rates at 9 months after surgery and later were significantly higher than the recovery rate at 3 months after surgery (Table 2).

Surgical outcome at the final follow-up was good in 15 patients (Cases 2, 3, 4, 6, 8, 10, 11, 13, 14, 16, 19, 20, 21, 22, and 24) and fair in 9 patients (Cases 1, 5, 7, 9, 12, 15, 17, 18, and 23). No patient was unchanged or worsened.

The JOA score reached its peak value at 3 months after surgery in 3 patients (Cases 5, 9, 13), at 6 months after surgery in 7 patients (Cases 3, 7, 10, 12, 16, 18, 23), at 9 months after surgery in 4 patients (Cases 1, 4, 8, 21), at 12 months after surgery in 7 patients (Cases 2, 6, 14, 15, 17, 20, 22), at 24 months in 2 patients (Cases 11, 19), and at the final follow-up in 1 patient (Case 24). The median time point that the JOA score reached its peak value was 9 months after surgery.

No patient chose additional anterior decompression surgery via thoracotomy.

Complications

In the present series, no patient developed persistent paralysis after surgery, but one patient (3.8%) had transient paralysis immediately after surgery (Case 23). Cerebrospinal fluid leakage occurred after laminectomy in one patient (3.8%) (Case 6). No instrumented failures occurred, such as PS loosening, hook displacement, or rod breakage.

Kyphotic angles at instrumented fusion levels

In the last six consecutive patients (Case 19 through Case 24), the preoperative kyphotic angles at the instrumented fusion levels were measured in the supine, prone, and sitting positions (Table 3). The difference between the kyphotic angle in the supine position and the kyphotic angle in the sitting position ranged from 8° (Case 20) to 20° (Case 24), indicating that some mobility remained in the thoracic spine in spite of the presence of OPLL. The mean spinal mobility per disc was 1.3°, ranging from 0.9° to 2.0°.

In all six patients, the postoperative kyphotic angle at the instrumented fusion levels was greater than the preoperative kyphotic angle in the supine position, but less than the preoperative kyphotic angle in the sitting position. Evaluating the correction of kyphosis after surgery with respect to the preoperative kyphotic angle in the sitting position demonstrated some correction of the kyphosis, with a mean change in kyphotic angle of 3.2° (Table 4). However, evaluating the correction of kyphosis after surgery with respect to the preoperative kyphotic angle in the supine position demonstrated increase of the kyphosis, with a mean change in kyphotic angle of −7.8° (Table 4). Figure 1 presents an illustrative example (Case 20) of the change in kyphotic angle following PDF, as demonstrated by a comparison of preoperative (b–d) and postoperative (f) radiographs.

Intraoperative ultrasonography

Intraoperative ultrasonography demonstrated that the decompression status for all 24 patients was the contact type, indicating that the posterior shift of the spinal cord

Table 3 Kyphotic angles at instrumented fusion levels

Case no.	Instrumented fusion levels/(no. of discs)	Preop. kyphotic angle (°)			Spinal mobility (°)	Spinal mobility per disc (°)	Postop. kyphotic angle (°)
		Supine position	Prone position	Sitting position			
19	T6–L2/(8)	15	23	25	10	1.3	23
20	T1–T10/(9)	27	32	35	8	0.9	32
21	T1–L1/(12)	40	47	51	11	0.9	49
22	T1–T10/(11)	23	26	34	11	1.2	32
23	T2–T10/(8)	30	37	40	10	1.3	38
24	T1–T11/(10)	25	33	45	20	2.0	37
Average ± SD		26.7 ± 1.3	33.0 ± 8.5	38.3 ± 9.1	11.7 ± 4.2	1.3 ± 0.4	35.2 ± 8.6

Spinal mobility = Preop. kyphotic angle in sitting position − preop. kyphotic angle in supine position

Table 4 Correction of kyphosis at instrumented fusion levels

Case no.	Preop. kyphotic angle at supine position − postop. kyphotic angle (°)	Preop. kyphotic angle at prone position − postop. kyphotic angle (°)	Preop. kyphotic angle at sitting position − postop. kyphotic angle (°)
19	−8	0	2
20	−5	0	3
21	−9	−2	2
22	−9	−6	2
23	−8	−1	2
24	−8	−4	8
Average ± SD	−7.8 ± 1.5	−2.2 ± 2.4	3.2 ± 2.4

was insufficient to prevent persistent impingement of the spinal cord from the anterior direction after laminectomy.

Discussion

Mechanisms for the improvement of myelopathy

The results of this study demonstrated that all patients showed neurological recovery after PDF at the final follow-up, with an average recovery rate of 58.1%. Compared with the results from previous published reports regarding thoracic OPLL, the surgical outcome of PDF was superior to the surgical outcome of laminectomy alone [5, 19]. When laminectomy alone is performed for thoracic OPLL, the backward shift of the spinal cord is often restricted because the thoracic spine is physiologically kyphotic, leading to persistent anterior impingement of the spinal cord by OPLL. In the present study, we evaluated the posterior shift of the spinal cord after laminectomy using intraoperative sonography and observed persistent anterior impingement of the spinal cord by OPLL in all cases. Despite this insufficient decompression of the spinal cord, PDF did result in considerable neurological recovery, indicating that posterior instrumented fusion has some positive effect on myelopathy after laminectomy for thoracic OPLL.

In this study, we also demonstrated that, in patients with thoracic OPLL, the spinal column still showed some mobility at the cord compression level in spite of the presence of massive heterotopic vertebral ossification. Our previous studies on patients with cervical myelopathy due to OPLL have shown that hypermobility of the vertebra at the cord compression level is a risk factor for the development and aggravation of myelopathy [11] and for poor surgical outcome after laminoplasty [7]. Taken together with the present findings, our investigations suggest that the remaining mobility of the spinal column at the cord compression level correlates with the development and aggravation of myelopathy in patients with thoracic OPLL.

Regarding correction of kyphosis by posterior instrumented fusion, we measured kyphotic angles in our last six cases. In all six cases, the postoperative kyphotic angle was smaller than the preoperative kyphotic angle in the supine position but greater than the preoperative kyphotic angle in the sitting position. However, even if we based our calculation of the correction of kyphosis upon the preoperative kyphotic angle in the sitting position, the mean correction still was only 3.2°, indicating that our procedure of posterior instrumented fusion did not sufficiently correct our patients' kyphosis. In turn, these results suggest that correction of kyphosis is not a major factor contributing to the neurological improvements observed after PDF.

In the present study, the JOA scores reached a peak value from 3 to 24 months after PDF (median 9 months), indicating that improvement of myelopathy in our patients was slowly progressed. Our findings suggest that suppression of spinal column mobility by posterior instrumented fusion is a more powerful factor than correction of kyphosis for producing neurological recovery after PDF. After PDF, anterior impingement of the spinal cord by OPLL persists, but the stabilization of the spine appears to decrease the damage to the spinal cord at the cord compression level, enabling a slow neurological recovery to commence.

Risk of postoperative paralysis

In our earlier study of 17 patients who underwent PDF for thoracic OPLL, no postoperative paralysis occurred after PDF [18]. Based on this indication that PDF entailed a low risk of postoperative neurological deterioration, we have employed PDF for all cases of thoracic OPLL treated surgically at our institute since 2003. However, we did encounter our first case of postoperative paralysis with our 23rd patient. Fortunately, the paralysis spontaneously resolved without adding OPLL extirpation. This incident thus suggests that the decompression procedure itself in patients with a severely compressed spinal cord entails a

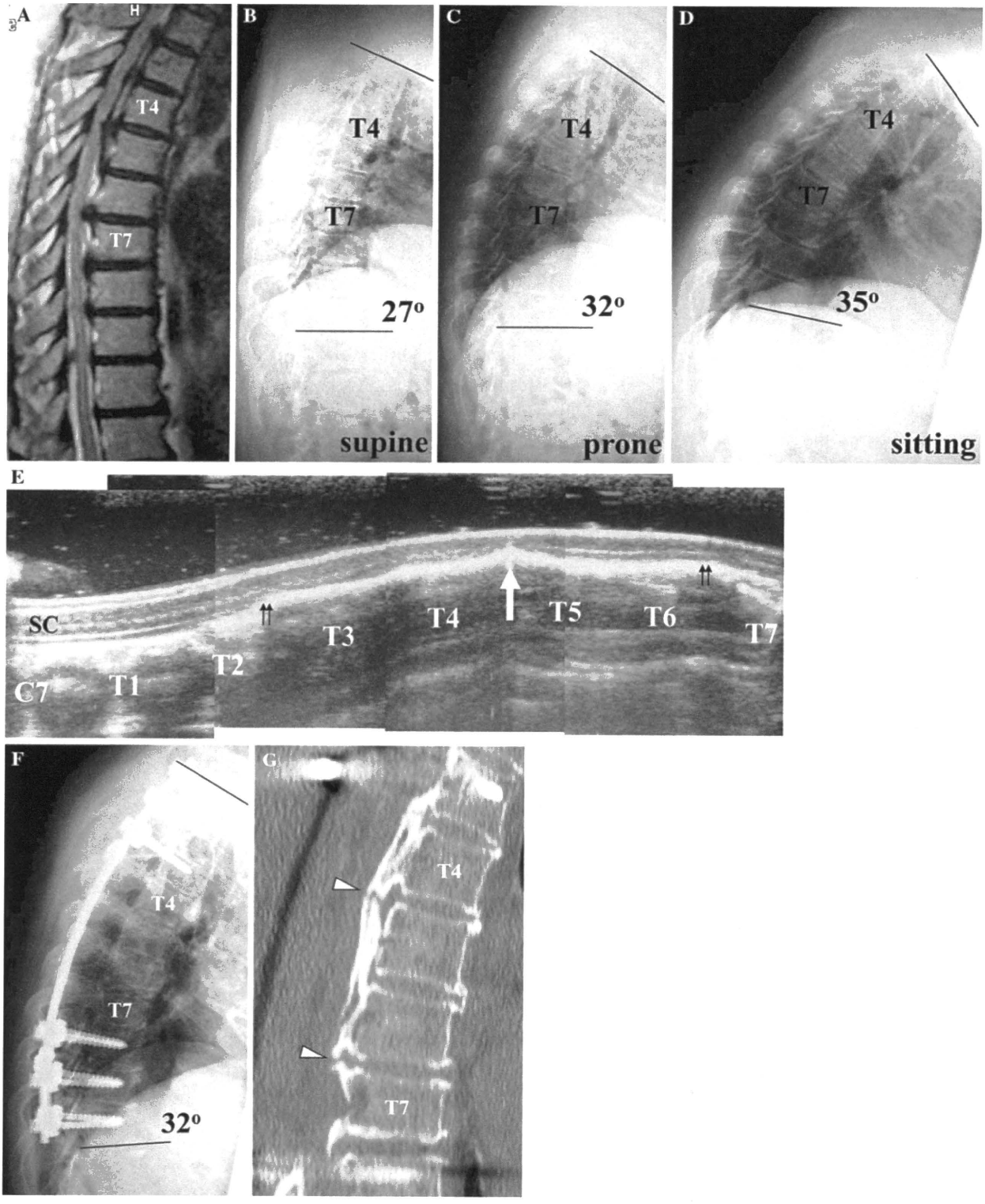

Fig. 1 Preoperative T2-weighted MR image at the midsagittal plane (**a**) of a 55-year-old woman (Case 20), showing severe narrowing of the spinal cord at T4/5 and T6/7. Preoperative radiographic images show that the kyphotic angle at T1–T10 was 27° in the supine position (**b**), 32° in the prone position (**c**) and 35° in the sitting position (**d**). Intraoperative spinal ultrasonography at the midsagittal plane after laminectomy shows anterior impingement of the spinal cord by a beak-type OPLL at T4/5 (**e**, *arrow*) and absence of the subarachnoid space on the ventral side of the spinal cord from the T2/3 to T6/7 levels (**e**, *double arrows*). *SC* spinal cord. A postoperative radiographic image (**f**) shows a kyphotic angle at T1–T10 of 32°. A midsagittal reconstruction CT image (**g**) shows a non-ossified area at the mid-portion of the ossified mass at T4/5 and T6/7 (**g**, *arrowheads*)

risk of postoperative paralysis, such that even the selection of PDF as our surgical procedure for thoracic OPLL cannot completely eliminate the risk of postoperative paralysis. However, in light of what appears to be a higher risk of postoperative paralysis following other surgical procedures such as laminectomy alone [5, 19] and OPLL extirpation [2, 4, 10, 13], we would suggest that PDF is the safest surgical procedure among the alternatives for thoracic OPLL.

Indication of additional OPLL extirpation

We have employed the same concepts in planning PDF as we use in planning circumspinal decompression [6, 15]. We explained to patients that PDF was the first operation and that we could add OPLL extirpation via thoracotomy as the second operation if their neurological recovery after PDF was insufficient. At our institute, the informed consent procedure for patients undergoing the first operation included our presenting all the information we had about the advantages and the disadvantages of additional OPLL extirpation surgery, including the high neurological recovery rate in successful cases of OPLL extirpation as well as the attendant risk of postoperative deterioration [6, 13, 18]. The choice of adding a second surgery was then left to the patient. All 24 patients in the present series have been sufficiently satisfied with the surgical outcome obtained by PDF alone such that no patient to date has elected additional anterior decompression surgery over a mean postoperative follow-up of 4 years and 5 months. Although all the patients understand the likelihood of much better neurological recovery after the addition of OPLL extirpation, thus far they appear to prefer not subjecting themselves to a new risk of postoperative paralysis that a second procedure would entail.

When neurological recovery after PDF is insufficient, we should consider adding anterior OPLL extirpation surgery. The findings from our patient group demonstrating gradual neurological recovery after PDF indicate that additional anterior surgery during the early stage of recovery after PDF generally is not desirable. In particular, since the patients' JOA scores reached their peak value at 9 months after PDF, we should follow postoperative neurological recovery in patients for at least 9 months to a year before arriving at a decision regarding additional anterior surgery.

Acknowledgments The authors thank Professor Kazuhisa Takahashi (Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine) for his kind support in the present analysis. This work was supported by the Health Labour Science Research Grant of Japan.

References

1. Ataka H, Tanno T, Yamazaki M (2009) Posterior instrumented fusion without neural decompression for incomplete neurological deficits following vertebral collapse in the osteoporotic thoracolumbar spine. Eur Spine J 18:69–76
2. Fujimura Y, Nishi Y, Nakamura M et al (1997) Long-term follow-up study of anterior decompression and fusion for thoracic myelopathy resulting from ossification of the posterior longitudinal ligament. Spine 22:305–311
3. Fujiyoshi T, Yamazaki M, Kawabe J et al (2008) A new concept for making decisions regarding the surgical approach for cervical ossification of the posterior longitudinal ligament: the K-line. Spine 33:E990–E993
4. Hanai K, Ogikubo O, Miyashita T (2002) Anterior decompression for myelopathy resulting from thoracic ossification of the posterior longitudinal ligament. Spine 27:1070–1076
5. Hasegawa K, Kaneda K, Abumi K, et al (1996) Operative indication of posterior decompression for thoracic ossification of the posterior longitudinal ligament. Investigation Committee Report on the Ossification of the Spinal Ligaments. Japanese Ministry of Health, Labour and Welfare, Tokyo, pp 305–309 (in Japanese)
6. Kawahara N, Tomita K, Murakami H et al (2008) Circumspinal decompression with dekyphosis stabilization for thoracic myelopathy due to ossification of the posterior longitudinal ligament. Spine 33:39–46
7. Masaki Y, Yamazaki M, Okawa A et al (2007) An analysis of factors causing poor surgical outcome in patients with cervical myelopathy due to ossification of the posterior longitudinal ligament: anterior decompression with spinal fusion versus laminoplasty. J Spinal Disord Tech 20:7–13
8. Matsumoto M, Chiba K, Toyama Y et al (2008) Surgical results and related factors for ossification of posterior longitudinal ligament of the thoracic spine: a multi-institutional retrospective study. Spine 33:1034–1041
9. Matsuyama Y, Yoshihara H, Tsuji T et al (2005) Surgical outcome of ossification of the posterior longitudinal ligament (OPLL) of the thoracic spine: implication of the type of ossification and surgical options. J Spinal Disord Tech 18:492–497
10. Min JH, Jang JS, Lee SH (2008) Clinical results of ossification of the posterior longitudinal ligament (OPLL) of the thoracic spine treated by anterior decompression. J Spinal Disord Tech 21:116–119
11. Mochizuki M, Aiba A, Hashimoto M et al (2009) Cervical myelopathy in patients with ossification of the posterior longitudinal ligament. J Neurosurg Spine 10:122–128
12. Ohtani K, Nakai S, Fujimura Y et al (1982) Anterior surgical decompression for thoracic myelopathy as a result of ossification of the posterior longitudinal ligament. Clin Orthop 166:82–88
13. Takahata M, Ito M, Abumi K et al (2008) Clinical results and complications of circumferential spinal cord decompression through a single posterior approach for thoracic myelopathy caused by ossification of posterior longitudinal ligament. Spine 33:1199–1208
14. Tokuhashi Y, Matsuzaki H, Oda H et al (2006) Effectiveness of posterior decompression for patients with ossification of the posterior longitudinal ligament in the thoracic spine: usefulness of the ossification-kyphosis angle on MRI. Spine 31:E26–E30
15. Tomita K, Kawahara N, Baba H et al (1990) Circumspinal decompression for thoracic myelopathy due to combined ossification of the posterior longitudinal ligament and ligamentum flavum. Spine 15:1114–1120
16. Yamazaki M, Koda M, Okawa A et al (2006) Transient paraparesis after laminectomy for thoracic ossification of the posterior longitudinal ligament and ossification of the ligamentum flavum. Spinal Cord 44:130–134

17. Yamazaki M, Okawa A, Koda M et al (2005) Transient paraparesis after laminectomy for thoracic myelopathy due to ossification of the posterior longitudinal ligament: a case report. Spine 30:E343–E346
18. Yamazaki M, Mochizuki M, Ikeda Y et al (2006) Clinical results of surgery for thoracic myelopathy caused by ossification of the posterior longitudinal ligament: operative indication of posterior decompression with instrumented fusion. Spine 31:1452–1460
19. Yonenobu K, Ebara S, Fujiwara K et al (1987) Thoracic myelopathy secondary to ossification of the spinal ligament. J Neurosurg 66:511–518

Journal of Clinical Neuroscience xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Case Report

Postoperative paralysis following posterior decompression with instrumented fusion for thoracic myelopathy caused by ossification of the posterior longitudinal ligament

Masashi Yamazaki, Akihiko Okawa, Chikato Mannoji, Takayuki Fujiyoshi, Takeo Furuya, Masao Koda

Spine Section, Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8677, Japan

ARTICLE INFO

Article history:
Received 17 January 2010
Accepted 5 April 2010
Available online xxxx

Keywords:
Decompression
Instrumented fusion
Ossification of posterior longitudinal ligament
Paralysis
Thoracic myelopathy

ABSTRACT

A 60-year-old man presented with thoracic myelopathy due to ossification of the posterior longitudinal ligament (OPLL). His spinal cord was severely impinged anteriorly by a beak-type OPLL and posteriorly by ossification of the ligamentum flavum at T4/5. He underwent surgical posterior decompression with instrumented fusion (PDF). Immediately after surgery, he developed a Brown-Séquard-type paralysis, which spontaneously resolved without requiring the addition of OPLL extirpation. This example highlights that the risk of postoperative neurological deterioration cannot be eliminated even when PDF is selected as the surgical procedure for thoracic OPLL, especially in instances in which the spinal cord is severely compressed.

1. Introduction

Despite the variety of surgical procedures for treating thoracic myelopathy due to ossification of the posterior longitudinal ligament (OPLL), postoperative paraplegia remains a major risk.[1,2] Twenty years ago, we hypothesized that stabilizing the spine with instrumentation could yield a certain degree of neurological recovery even without complete OPLL extirpation. Based on this hypothesis, in 1989 we introduced the surgical procedure of posterior decompression with instrumented fusion (PDF) for patients with thoracic OPLL. In our earlier study of 17 patients who underwent PDF for thoracic OPLL, all of the patients had a considerable degree of neurological recovery and no postoperative paralysis occurred.[3] Based on the results of that study, we have employed PDF for all instances of thoracic OPLL treated surgically at our institute since 2003. Subsequently, however, we had our first encounter with postoperative paralysis in our 23rd PDF patient.

2. Case report

A 60-year-old man had thoracic myelopathy with a Japanese Orthopaedic Association (JOA) score of 4.5, on a scale from 0 to 11. He complained of bilateral motor weakness of his lower extremities and was unable to walk without a cane. Lateral radiographs showed T4–9 OPLL. MRI scans showed severe narrowing of the spinal cord at T4/5. Reconstruction images from a CT myelogram showed impingement of the spinal cord anteriorly by a beak-type OPLL (Fig. 1) and posteriorly by ossification of the ligamentum flavum (OLF) (Fig. 1A) at T4/5. The image showing a beak-type OPLL also showed a non-ossified area between the ossified masses at T4/5 (Fig. 1A), indicating that the spinal column still had some mobility at the cord compression level.

We performed a T4–7 laminectomy and a T2–10 posterior instrumented fusion using pedicle screws as anchors at the T2, T3, T4, T8, T9 and T10 levels. An intraoperative spinal ultrasonography after the laminectomy showed continuing anterior impingement of the spinal cord by the beak-type OPLL at T4/5 and an absence of the subarachnoid space on the ventral side of the spinal cord (Fig. 2).

Immediately after surgery, the patient suffered a severe motor loss of his right lower limb (muscle strength at grade 0 out of 5) and analgesia of his left lower extremity and left trunk below the umbilicus, indicative of a Brown-Séquard-type paralysis. We immediately administered a 1000 mg bolus of methylprednisolone sodium succinate intravenously. One hour after surgery, we detected muscle contraction in the patient's right lower limb. The morning following surgery, his right lower limb showed a slight recovery of motor function (muscle strength at grade 1 to 3 out of 5). By the end of the day, the sensory loss in his left lower extremity and trunk had begun to diminish, showing hypalgesia at grade 5 to 8 out of 10. His neurological recovery gradually pro-

Corresponding author. Tel.: +81 43 226 2117; fax: +81 43 226 2116.
E-mail address: masashiy@faculty.chiba-u.jp (M. Yamazaki).

0967-5868/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jocn.2010.04.030

Please cite this article in press as: Yamazaki M et al. Postoperative paralysis following posterior decompression with instrumented fusion for thoracic myelopathy caused by ossification of the posterior longitudinal ligament. *J Clin Neurosci* (2010), doi:10.1016/j.jocn.2010.04.030

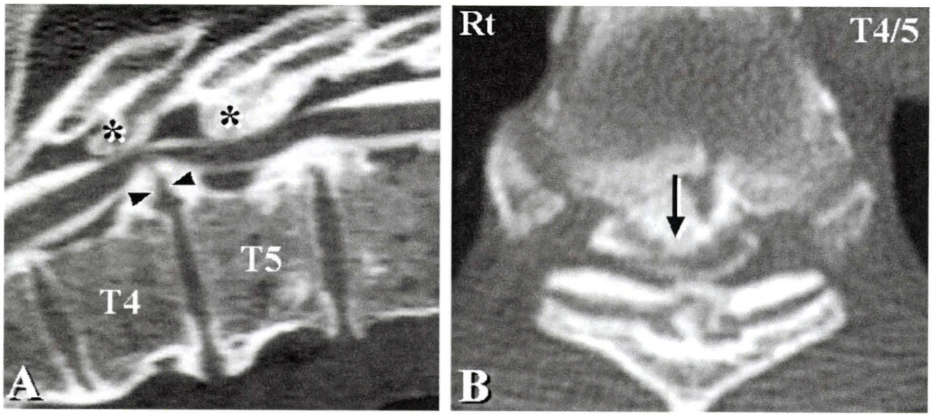

Fig. 1. Preoperative midsagittal (A) and axial (B) reconstruction images from a CT myelogram showing impingement of the spinal cord anteriorly by a beak-type ossification of the posterior longitudinal ligament (OPLL) (A, B, arrow) and posteriorly by ossification of the ligamentum flavum (A, asterisks) at T4/5. The mid-portion of the beak-type OPLL contains a non-ossified area (A, arrowheads).

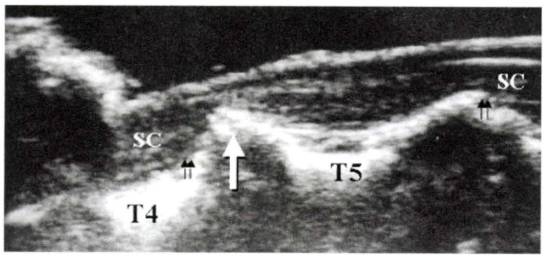

Fig. 2. Intraoperative spinal ultrasonography in the midsagittal plane after laminectomy showing anterior impingement of the spinal cord (SC) by the beak-type ossification of the posterior longitudinal ligament at T4/5 (arrow) and the absence of the subarachnoid space on the ventral side of the spinal cord from T4/5 to T5/6 (double arrows).

gressed from that point on. Three months after surgery, the patient could walk with a cane. Six months after surgery, his JOA score had risen to 7, and his recovery was 38.5%. At the final follow-up 5 years and 3 months after surgery, the patient could walk without a cane and his recovery remained at 38.5%.

3. Discussion

During PDF, we pay maximal attention to avoid injury to the spinal cord.[3] In spite of such efforts, neurological deterioration occurred immediately after surgery in this patient. This incident suggests that the decompression procedure itself entails a risk of postoperative paralysis in patients with a severely compressed spinal cord. It is possible that when the spinal cord is severely compressed by OPLL and OLF from both anterior and posterior directions, as in our patient, the risk of intraoperative injury to the spinal cord may increase. In instances with a severely pinched spinal cord, the risk of postoperative neurological deterioration evidently cannot be completely eliminated, even when PDF is selected as the surgical procedure for thoracic OPLL.

An alternative treatment option for this patient would have been single-stage anterior and posterior decompression for combined thoracic OPLL and OLF. Several authors have previously reported excellent clinical results using this procedure.[1,4] However, in the same papers, these authors also reported several examples of postoperative paralysis after single-stage anterior and posterior compression, which suggests that the overall outcomes of single-stage anterior and posterior decompression are not necessarily superior to the outcomes of PDF.[1,4]

Published studies have also shown that anterior decompression through thoracotomy does not necessarily produce favorable results when performed as rescue surgery on thoracic OPLL and OLF patients whose myelopathy worsens after laminectomy.[5,6] Of particular concern is the possibility that worsening myelopathy might indicate severe damage to the spinal cord resulting from the laminectomy, in which case the spinal cord may likely not further tolerate an anterior decompression procedure. Because of this risk, we did not choose anterior extirpation of OPLL through thoracotomy as rescue surgery in our patient.

Fortunately, our patient's paralysis spontaneously resolved without requiring us to add OPLL extirpation. In light of what appears to be a higher risk of postoperative paralysis following other surgical procedures such as laminectomy alone and OPLL extirpation,[1,2] we suggest that PDF is still the safest surgical procedure among the surgical treatment alternatives for thoracic OPLL. To further improve the safety of PDF for thoracic OPLL, however, we will need to further modify the treatment protocol to reduce the risk of damage to the spinal cord during PDF. One promising possibility is neuroprotective therapy with preoperative administration of neural growth factors.

Acknowledgement

This work was supported by a Health Labour Science Research Grant of Japan.

References

1. Takahata M, Ito M, Abumi K, et al. Clinical results and complications of circumferential spinal cord decompression through a single posterior approach for thoracic myelopathy caused by ossification of posterior longitudinal ligament. *Spine* 2008;**33**:1199–208.
2. Matsumoto M, Chiba K, Toyama Y, et al. Surgical results and related factors for ossification of posterior longitudinal ligament of the thoracic spine: a multi-institutional retrospective study. *Spine* 2008;**33**:1034–41.
3. Yamazaki M, Mochizuki M, Ikeda Y, et al. Clinical results of surgery for thoracic myelopathy caused by ossification of the posterior longitudinal ligament: operative indication of posterior decompression with instrumented fusion. *Spine* 2006;**31**:1452–60.
4. Tomita K, Kawahara N, Baba H, et al. Circumspinal decompression for thoracic myelopathy due to combined ossification of the posterior longitudinal ligament and ligamentum flavum. *Spine* 1990;**15**:1114–20.
5. Ohtani K, Nakai S, Fujimura Y, et al. Anterior surgical decompression for thoracic myelopathy as a result of ossification of the posterior longitudinal ligament. *Clin Orthop* 1982;**166**:82–8.
6. Yonenobu K, Ebara S, Fujiwara K, et al. Thoracic myelopathy secondary to ossification of the spinal ligament. *J Neurosurg* 1987;**66**:511–8.

Please cite this article in press as: Yamazaki M et al. Postoperative paralysis following posterior decompression with instrumented fusion for thoracic myelopathy caused by ossification of the posterior longitudinal ligament. *J Clin Neurosci* (2010), doi:10.1016/j.jocn.2010.04.030

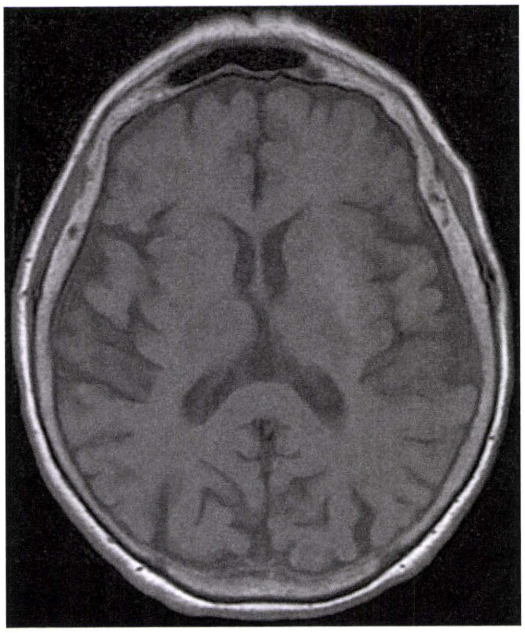

Fig. 2. Axial T1-weighted MRI at the same level shows hyperintensity of the left lentiform nucleus.

theories have been proposed to explain the imaging findings including calcium deposition, petechial haemorrhage, ischaemia, infarction, cytotoxic oedema and myelin breakdown. It is of note that this focal abnormality is induced by hyperglycaemia, a systemic disturbance. Pre-existing basal ganglia disease, such as focal small vessel ischaemia, may be present in these diabetic patients, with hyperglycaemia tipping the balance in these regions into cellular dysfunction. Hyperglycaemia impairs cerebral autoregulation, which may explain hypoperfusion on imaging, and causes gamma-aminobutyric acid (GABA) depletion in basal ganglia neurons as a result of anaerobic metabolism.[6] It may be the GABA depletion, the main inhibitory neurotransmitter in the basal ganglia, which results in chorea. Although much further investigation is needed, the pathological and radiological findings suggest that non-ketotic hyperglycaemic crisis induces ischaemia and metabolic disturbance resulting in basal ganglia dysfunction.

References

1. Oh S, Lee K, Im J, et al. Chorea associated with non-ketotic hyperglycemia and hyperintensity basal ganglia lesion on T1-weighted brain MRI study: a meta-analysis of 53 cases including four present cases. *J Neurol Sci* 2002;**200**:57–62.
2. Fujioka M, Taoka K, Matsuo Y, et al. Magnetic resonance imaging shows delayed ischemic striatal neurodegeneration. *Ann Neurol* 1998;**54**:732–47.
3. Shan D, Ho D, Chang C, et al. Hemichorea-hemiballism an explanation for MR signal changes. *Am J Neuroradiol* 1998;**19**:863–70.
4. Lai P, Tien RD, Chang M, et al. Chorea-ballismus with nonketotic hyperglycemia in primary diabetes mellitus. *Am J Neuroradiol* 1996;**17**:1057–64.
5. Hsu JL, Wang H, Hsu W. Hyperglycemia-induced unilateral basal ganglion lesions with and without hemichorea. A PET study. *J Neurol* 2004;**251**:1486–90.
6. Cherian A, Thomas B, Neeraj NB, et al. Concepts and controversies in nonketotic hyperglycaemia-induced hemichorea: further evidence from susceptibility-weighted MR imaging. *J Magn Reson Imaging* 2009;**29**:699–703.

doi:10.1016/j.jocn.2010.04.036

Postoperative paralysis following posterior decompression with instrumented fusion for thoracic myelopathy caused by ossification of the posterior longitudinal ligament

Masashi Yamazaki *, Akihiko Okawa, Chikato Mannoji, Takayuki Fujiyoshi, Takeo Furuya, Masao Koda

Spine Section, Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8677, Japan

ARTICLE INFO

Article history:
Received 17 January 2010
Accepted 5 April 2010

Keywords:
Decompression
Instrumented fusion
Ossification of posterior longitudinal ligament
Paralysis
Thoracic myelopathy

ABSTRACT

A 60-year-old man presented with thoracic myelopathy due to ossification of the posterior longitudinal ligament (OPLL). His spinal cord was severely impinged anteriorly by a beak-type OPLL and posteriorly by ossification of the ligamentum flavum at T4/5. He underwent surgical posterior decompression with instrumented fusion (PDF). Immediately after surgery, he developed a Brown-Séquard-type paralysis, which spontaneously resolved without requiring the addition of OPLL extirpation. This example highlights that the risk of postoperative neurological deterioration cannot be eliminated even when PDF is selected as the surgical procedure for thoracic OPLL, especially in instances in which the spinal cord is severely compressed.

1. Introduction

Despite the variety of surgical procedures for treating thoracic myelopathy due to ossification of the posterior longitudinal liga-

* Corresponding author. Tel.: +81 43 226 2117; fax: +81 43 226 2116.
E-mail address: masashiy@faculty.chiba-u.jp (M. Yamazaki).

ment (OPLL), postoperative paraplegia remains a major risk.[1,2] Twenty years ago, we hypothesized that stabilizing the spine with instrumentation could yield a certain degree of neurological recovery even without complete OPLL extirpation. Based on this hypothesis, in 1989 we introduced the surgical procedure of posterior decompression with instrumented fusion (PDF) for patients with thoracic OPLL. In our earlier study of 17 patients who underwent PDF for thoracic OPLL, all of the patients had a considerable degree of neurological recovery and no postoperative paralysis occurred.[3] Based on the results of that study, we have employed PDF for all instances of thoracic OPLL treated surgically at our institute since 2003. Subsequently, however, we had our first encounter with postoperative paralysis in our 23rd PDF patient.

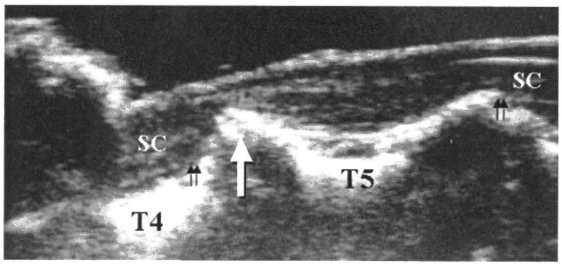

Fig. 2. Intraoperative spinal ultrasonography in the midsagittal plane after laminectomy showing anterior impingement of the spinal cord (SC) by the beak-type ossification of the posterior longitudinal ligament at T4/5 (arrow) and the absence of the subarachnoid space on the ventral side of the spinal cord from T4/5 to T5/6 (double arrows).

2. Case report

A 60-year-old man had thoracic myelopathy with a Japanese Orthopaedic Association (JOA) score of 4.5, on a scale from 0 to 11. He complained of bilateral motor weakness of his lower extremities and was unable to walk without a cane. Lateral radiographs showed T4–9 OPLL. MRI scans showed severe narrowing of the spinal cord at T4/5. Reconstruction images from a CT myelogram showed impingement of the spinal cord anteriorly by a beak-type OPLL (Fig. 1) and posteriorly by ossification of the ligamentum flavum (OLF) (Fig. 1A) at T4/5. The image showing a beak-type OPLL also showed a non-ossified area between the ossified masses at T4/5 (Fig. 1A), indicating that the spinal column still had some mobility at the cord compression level.

We performed a T4–7 laminectomy and a T2–10 posterior instrumented fusion using pedicle screws as anchors at the T2, T3, T4, T8, T9 and T10 levels. An intraoperative spinal ultrasonography after the laminectomy showed continuing anterior impingement of the spinal cord by the beak-type OPLL at T4/5 and an absence of the subarachnoid space on the ventral side of the spinal cord (Fig. 2).

Immediately after surgery, the patient suffered a severe motor loss of his right lower limb (muscle strength at grade 0 out of 5) and analgesia of his left lower extremity and left trunk below the umbilicus, indicative of a Brown-Séquard-type paralysis. We immediately administered a 1000 mg bolus of methylprednisolone sodium succinate intravenously. One hour after surgery, we detected muscle contraction in the patient's right lower limb. The morning following surgery, his right lower limb showed a slight recovery of motor function (muscle strength at grade 1 to 3 out of 5). By the end of the day, the sensory loss in his left lower extremity and trunk had begun to diminish, showing hypalgesia at grade 5 to 8 out of 10. His neurological recovery gradually progressed from that point on. Three months after surgery, the patient could walk with a cane. Six months after surgery, his JOA score had risen to 7, and his recovery was 38.5%. At the final follow-up 5 years and 3 months after surgery, the patient could walk without a cane and his recovery remained at 38.5%.

3. Discussion

During PDF, we pay maximal attention to avoid injury to the spinal cord.[3] In spite of such efforts, neurological deterioration occurred immediately after surgery in this patient. This incident suggests that the decompression procedure itself entails a risk of postoperative paralysis in patients with a severely compressed spinal cord. It is possible that when the spinal cord is severely compressed by OPLL and OLF from both anterior and posterior directions, as in our patient, the risk of intraoperative injury to the spinal cord may increase. In instances with a severely pinched spinal cord, the risk of postoperative neurological deterioration evidently cannot be completely eliminated, even when PDF is selected as the surgical procedure for thoracic OPLL.

An alternative treatment option for this patient would have been single-stage anterior and posterior decompression for combined thoracic OPLL and OLF. Several authors have previously reported excellent clinical results using this procedure.[1,4] However, in the same papers, these authors also reported several examples of postoperative paralysis after single-stage anterior and posterior compression, which suggests that the overall outcomes of single-stage anterior and posterior decompression are not necessarily superior to the outcomes of PDF.[1,4]

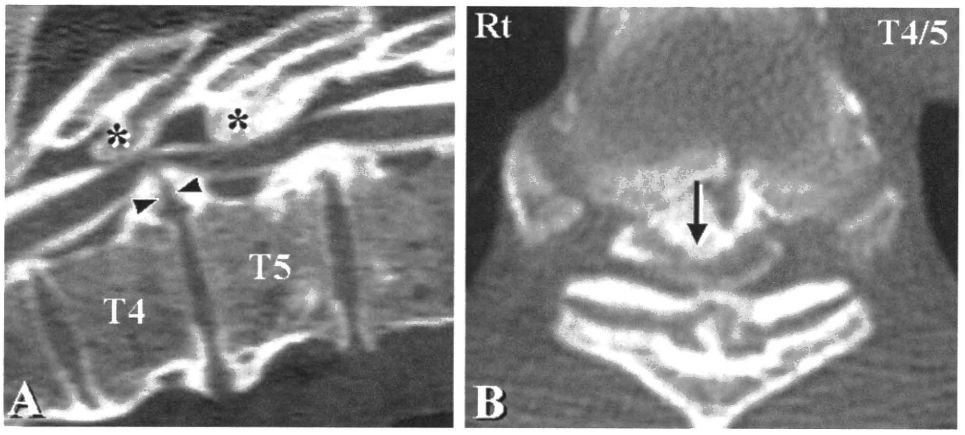

Fig. 1. Preoperative midsagittal (A) and axial (B) reconstruction images from a CT myelogram showing impingement of the spinal cord anteriorly by a beak-type ossification of the posterior longitudinal ligament (OPLL) (A, B, arrow) and posteriorly by ossification of the ligamentum flavum (A, asterisks) at T4/5. The mid-portion of the beak-type OPLL contains a non-ossified area (A, arrowheads).

Published studies have also shown that anterior decompression through thoracotomy does not necessarily produce favorable results when performed as rescue surgery on thoracic OPLL and OLF patients whose myelopathy worsens after laminectomy.[5,6] Of particular concern is the possibility that worsening myelopathy might indicate severe damage to the spinal cord resulting from the laminectomy, in which case the spinal cord may likely not further tolerate an anterior decompression procedure. Because of this risk, we did not choose anterior extirpation of OPLL through thoracotomy as rescue surgery in our patient.

Fortunately, our patient's paralysis spontaneously resolved without requiring us to add OPLL extirpation. In light of what appears to be a higher risk of postoperative paralysis following other surgical procedures such as laminectomy alone and OPLL extirpation,[1,2] we suggest that PDF is still the safest surgical procedure among the surgical treatment alternatives for thoracic OPLL. To further improve the safety of PDF for thoracic OPLL, however, we will need to further modify the treatment protocol to reduce the risk of damage to the spinal cord during PDF. One promising possibility is neuroprotective therapy with preoperative administration of neural growth factors.

Acknowledgement

This work was supported by a Health Labour Science Research Grant of Japan.

References

1. Takahata M, Ito M, Abumi K, et al. Clinical results and complications of circumferential spinal cord decompression through a single posterior approach for thoracic myelopathy caused by ossification of posterior longitudinal ligament. *Spine* 2008;**33**:1199–208.
2. Matsumoto M, Chiba K, Toyama Y, et al. Surgical results and related factors for ossification of posterior longitudinal ligament of the thoracic spine: a multiinstitutional retrospective study. *Spine* 2008;**33**:1034–41.
3. Yamazaki M, Mochizuki M, Ikeda Y, et al. Clinical results of surgery for thoracic myelopathy caused by ossification of the posterior longitudinal ligament: operative indication of posterior decompression with instrumented fusion. *Spine* 2006;**31**:1452–60.
4. Tomita K, Kawahara N, Baba H, et al. Circumspinal decompression for thoracic myelopathy due to combined ossification of the posterior longitudinal ligament and ligamentum flavum. *Spine* 1990;**15**:1114–20.
5. Ohtani K, Nakai S, Fujimura Y, et al. Anterior surgical decompression for thoracic myelopathy as a result of ossification of the posterior longitudinal ligament. *Clin Orthop* 1982;**166**:82–8.
6. Yonenobu K, Ebara S, Fujiwara K, et al. Thoracic myelopathy secondary to ossification of the spinal ligament. *J Neurosurg* 1987;**66**:511–8.

doi:10.1016/j.jocn.2010.04.030

Fourth-ventricular immature teratoma

Sunil V. Furtado *, Nandita Ghosal, Vishal B. Rokade, Alangar S. Hegde

Department of Neurosurgery, Sri Sathya Sai Institute of Higher Medical Sciences, EPIP Area, Whitefield, Bangalore 560066, Karnataka State, India

ARTICLE INFO

Article history:
Received 11 March 2010
Accepted 28 March 2010

Keywords:
Chemotherapy
Fourth ventricle
Hydrocephalus
Immature teratoma
Posterior fossa

ABSTRACT

Teratomas account for 3% of all childhood tumors. This group of non-germinomatous germ cell tumors exhibit cellular and structural characteristics associated with the three germ layers. The immature cells can differentiate into more malignant neoplasms. We report the presentation and management of a 4-year-old girl with an immature teratoma of the fourth ventricle. The outcome of this intracranial immature teratoma was poor, due to the patient's age, the extensive lesion at presentation and the grade of the tumor.

1. Introduction

Intracranial teratomas represent 2% to 5% of all teratomas in infancy and account for 0.3% to 0.6% of all intracranial neoplasms.[1,2] They are seen involving midline intracranial structures; are more commonly reported in the pineal region, third ventricle and suprasellar region; and present with obstructive hydrocephalus.[1,3,4] Teratomas are extremely rare in the posterior fossa and are seldom reported in children beyond 2 years of age. We present a rare, large immature teratoma based exclusively in the fourth ventricle.

2. Case report

A 4-year-old girl with a 2-month history of headaches and vomiting presented with two episodes of hydrocephalus. She had left-side abducens palsy and florid papilledema. Her head circumference was normal for her age. A CT scan of her head revealed an isodense lesion in the posterior fossa occupying the entire fourth ventricle and causing obstructive hydrocephalus, for which she underwent an emergency ventriculoperitoneal shunt (Fig. 1a). A MRI brain scan showed a lesion in the fourth ventricle arising from the vermis with mixed intensity on T1- and T2-weighted images, with cystic areas and exhibiting non-homogeneous contrast enhancement. The patient underwent a midline suboccipital craniotomy and decompression of the tumour through the vermis (Fig. 1b,c,d). The tumor was grey and soft to firm in consistency with areas of calcification. It was infiltrating the floor of the fourth ventricle and was merging imperceptibly with the vermis. Near-total decompression was achieved because of brainstem infiltration. A postoperative MRI scan showed residual tumor along the ventricular floor with minimal blood in the operative cavity (Fig. 2). Histopathological examination revealed an immature teratoma of the posterior fossa having glial components with immature neural

* Corresponding author. Tel.: +91 80 2841 6837; fax: +91 80 2841 1503.
E-mail address: sunilvf@gmail.com (S.V. Furtado).

SPINE Volume 35, Number 9, pp E359–E362

Intraoperative Spinal Subarachnoid Hematoma in a Patient With Cervical Ossification of the Posterior Longitudinal Ligament

Masashi Yamazaki, MD, PhD, Akihiko Okawa, MD, PhD, Takayuki Fujiyoshi, MD, PhD, Junko Kawabe, MD, PhD, Takeo Furuya, MD, PhD, Tamiyo Kon, MD, PhD, and Masao Koda, MD, PhD

Study Design. Case report.

Objective. To report a surgically treated case of cervical ossification of the posterior longitudinal ligament (OPLL), in which a spinal subarachnoid hematoma (SSAH) developed intraoperatively but was successfully treated.

Summary of Background Data. Previous reports have indicated that trauma, lumbar puncture, vascular lesions such as arteriovenous malformation, neoplastic lesions, and coagulopathy can cause SSAH. To the best of our knowledge, there has been no report that describes the occurrence of SSAH during anterior decompression surgery of the cervical spine.

Methods. A 52-year-old man with cervical myelopathy caused by OPLL underwent surgery for anterior decompression from C2/3 to C6/7. Immediately after the OPLL floating procedure, cerebrospinal fluid leakage and massive bleeding occurred at right edge of the OPLL at the C3–C4 level. After hemostasis, the dura mater at the C5–C6 levels bulged rapidly and became cyanotic. Intraoperative ultrasonographic images showed a high-intensity mass lesion on the ventral side of the spinal cord, indicating an intrathecal hematoma.

Results. We incised the dura, found the hematoma under the intact arachnoid, and removed it. We then found that the bleeding occurred from the radicular artery along the right C4 root. After hemostasis, we performed anterior spine fusion from C2–C7. After surgery, the patient's myelopathy was improved, and no neurologic deficit related to the subarachnoid hematoma was found.

Conclusion. This experience suggests that when anterior decompression surgery is performed for cervical OPLL patients, we should consider the possible occurrence of an SSAH. Intraoperative ultrasonography is a useful tool for detecting SSAHs.

Key words: spinal subarachnoid hematoma, intraoperative ultrasonography, cervical spine, ossification of posterior longitudinal ligament, anterior decompression surgery. **Spine 2010;35:E359–E362**

From the Spine Section, Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, Chuo-Ku, Chiba, Japan.
Acknowledgment date: June 4, 2009. First revision date: September 22, 2009. Acceptance date: September 28, 2009.
The device(s)/drug(s) is/are FDA-approved or approved by corresponding national agency for this indication.
Funds were received in support of this work. No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.
Supported by a Japanese Health Labour Science Research Grant.
Address correspondence and reprint requests to Masashi Yamazaki, MD, PhD, Spine Section, Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-Ku, Chiba 260-8677, Japan; E-mail: masashiy@faculty.chiba-u.jp

Spinal subarachnoid hematoma (SSAH) occurs rarely. Previous reports have indicated that trauma, lumbar puncture, vascular lesions such as arteriovenous malformations, neoplastic lesions, and coagulopathy can cause SSAHs.[1–3] To the best of our knowledge, there has been no report that describes the occurrence of a SSAH during anterior decompression surgery of the cervical spine.

In the present study, we report a case of cervical myelopathy caused by ossification of the posterior longitudinal ligament (OPLL), in which SSAH developed during anterior decompression surgery. The SSAH was diagnosed intraoperatively using ultrasonography, and successfully treated.

Case Report

Clinical Profile

A 52-year-old man with a 12-month history of gait disturbance and clumsiness of bilateral hands was admitted to our institute. He could walk without support, but had a clumsy gait. He had motor weakness of both upper limbs, showing grade 4/5 muscle strength for finger extension and abduction. He had some sensory loss in his trunk and bilateral upper and lower extremities, showing grade 3–5/10 hypoalgesia. Hyper-reflexia was detected in both upper and lower extremities, and ankle clonus was transiently positive bilaterally. Hoffman, Wartenberg, and Babinski signs were positive bilaterally. He had no history of medication with anticoagulants. Routine blood tests, including coagulative parameters, were normal.

Radiologic examination demonstrated cervical OPLL at C3–C4. A midsagittal T2-weighted magnetic resonance image showed that the spinal cord was compressed anteriorly from C3/4 to C6/7 (Figure 1A). Sagittal and axial images from a computed tomographic myelogram revealed that the spinal cord was compressed by OPLL at C3–C4, had a soft disc herniation at C5/6, and an osteophyte at C6/7 (Figures 1B,C). On the sagittal image, the OPLL at C3–C4 exceeded the K-line, the line connecting the midpoints of the spinal canal at C2 and C7. Thus, this patient was classified into the K-line (−) group.[4]

Surgical Technique

We previously reported that, in the K-line (−) group, sufficient posterior shift of the spinal cord and neurologic improvement were not obtained after posterior decompression surgery.[4] Thus, the patient was prepared for

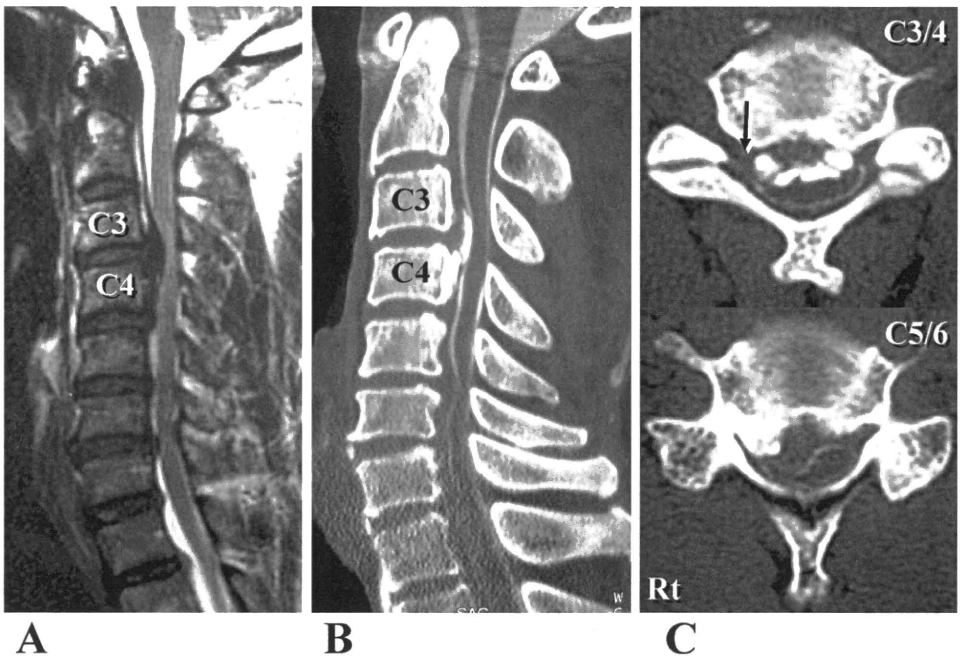

Figure 1. These 3 images of the cervical spine (**A–C**) were obtained before surgery in a 52-year-old man with cervical myelopathy caused by ossification of posterior longitudinal ligament (OPLL). A midsagittal T2-weighted magnetic resonance image (**A**) shows severe compression of the spinal cord anteriorly at multiple levels from C3/4 to C6/7. Midsagittal (**B**) and axial (**C**) computed tomographic images show anterior broad compression of the spinal cord by a large OPLL at C3/4 and anterior right-sided compression of the spinal cord by soft disc herniation at C5/6. An arrow (**C**, upper panel) indicates the site where cerebrospinal fluid leakage and subsequent massive bleeding occurred during surgery.

surgical treatment, which was planned as anterior decompression of the spinal cord and a strut bone graft with autologous fibula at C2–C7. At first, we performed corpectomy of C3, C4, C5, and C6, and extirpated the soft disc herniation at C5/6 and the osteophyte at C6/7. We then attempted to extirpate OPLL at C3–C4. However, the dura matter was ossified, and separation of OPLL from the dura was not possible. Therefore, we modified the surgical procedure from the extirpation of the OPLL to floating the OPLL.

We reduced the OPLL to be as thin as possible. Then, we cut the posterior longitudinal ligament around the thinned OPLL. When we finished cutting the posterior longitudinal ligament around the OPLL, cerebrospinal fluid (CSF) leakage and subsequent massive bleeding occurred at the right edge of the OPLL at the C3/4 level. We placed a collagen-impregnated sheet at the bleeding point, and compressed it with a cotton sheet. Hemostasis with this method was successful, and no further bleeding occurred there.

To confirm decompression of the spinal cord, we performed intraoperative ultrasonography. To our surprise, we found a high-intensity mass lesion at the ventral side of the spinal cord and at the cranial aspect of C5/6, indicating the development of an intrathecal hematoma that compressed the spinal cord (Figures 2A, B).

Immediately after we performed the ultrasonography, we found that the dura mater at the C5–C6 levels bulged and became deeply cyanotic (Figure 3, arrowhead). We considered that rapid growth of the hematoma had progressed and damage to the spinal cord may occur. Thus, we promptly cut the dura longitudinally at the C5–C6 levels to decompress the cord. We found that the arachnoid was intact, and beneath it there was a hematoma. We then cut through the arachnoid, and removed the subarachnoid hematoma.

To identify the bleeding point, we extirpated the OPLL at C3–C4. Because the dura mater was ossified at this region, a large defect of dura mater was formed after

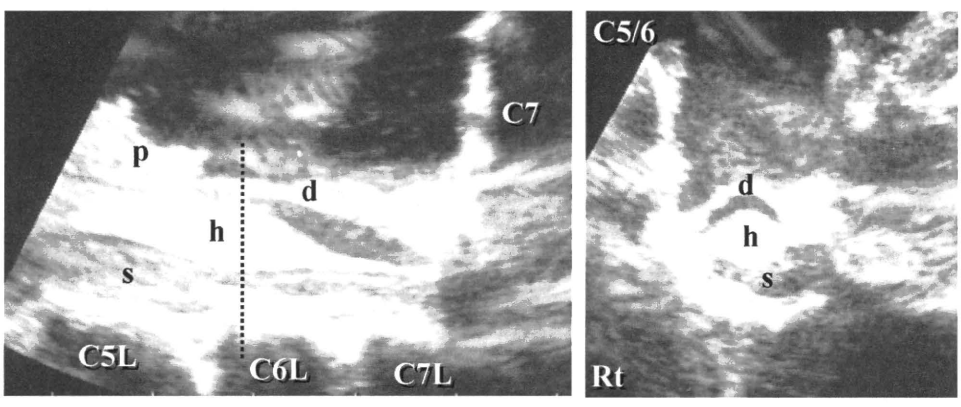

Figure 2. Intraoperative ultrasonographic images (**A, B**) immediately after the anterior decompression procedure from C2/3 to C6/7. A sagittal image slightly shifted to the right side (**A**) shows a high-intensity mass lesion at the ventral side of the spinal cord, indicating an intrathecal hematoma. A dotted line indicates the section depicted in (**B**). An axial image at the C5/6 level (**B**) shows the high-intensity mass lesion at the ventral and right sides of the spinal cord. h indicates intrathecal hematoma; s, spinal cord; d, dura matter; p, remained posterior longitudinal ligament; C4L, C5L, and C6L, lamina of C4, C5, and C6, respectively.

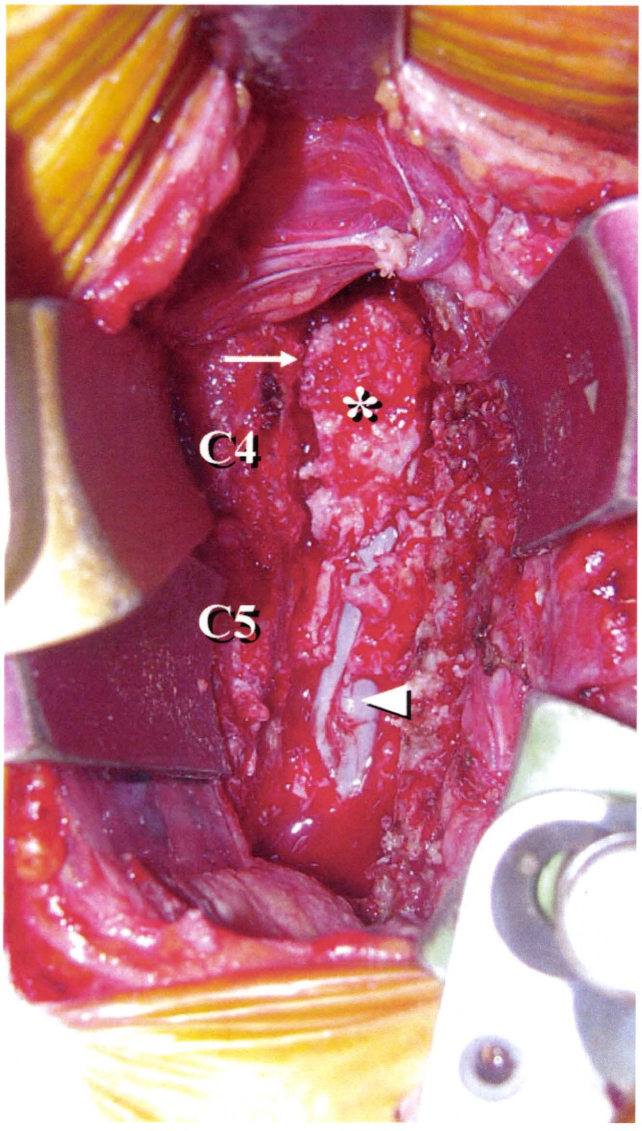

Figure 3. An intraoperative photograph after the anterior decompression from C2/3 to C6/7. Immediately after the OPLL floating procedure, cerebrospinal fluid leakage and massive bleeding occurred at the right edge of the OPLL at C3/4 level (arrow). After the hemostasis, the dura mater at the C5–C6 levels rapidly bulged and became deeply cyanotic (arrowhead). The asterisk indicates the floated OPLL at the C3–C4 levels.

extirpation of the OPLL. After this, we found that the point of bleeding was the radicular artery along the right C4 root (Figure 3, arrow). We coagulated the bleeding point, and completed the hemostasis. We removed the hematoma at the C3–C4 levels, and completed the decompression of the spinal cord. We constructed a dural patch. We then performed a strut bone graft with autologous fibula from C2–C7. The incision was closed, and the patient returned to the recovery room.

Clinical Outcome

After surgery, the patient's myelopathy was improved, and no neurologic deficit related to the SSAH was found. Magnetic resonance images 1 month after surgery showed adequate decompression of the spinal cord. At follow-up 1 year after the surgery, the patient was completely healthy, and returned to his job.

■ Discussion

Domenicucci et al[2] reviewed 69 cases of SSAH, and summarized its etiologic factors. In 31 (33.3%) of 69 cases, the cause was a lumbar puncture that was performed for anesthesia, CSF control, or myelography. Twelve (17.3%) cases were classified as spontaneous. In 11 (15.9%) cases, SSAH was correlated to trauma. In 9 (13%) cases, SSAH occurred because of coagulopathies, such as from taking anticoagulant medication. Daentzer *et al*[5] reported a case of SSAH after spine surgery, in which misplacement of a screw at surgery for an odontoid fracture caused vertebral artery injury. This caused a pseudoaneurysm and subsequent rupture, leading to a fatal subarachnoid hemorrhage 4 days after surgery.[5] Aghi *et al*[1] reported a case of SSAH after high cervical myelography by C1–C2 lateral puncture. Five days after the myelography, the patient was diagnosed as having SSAH by computed tomography, and the subarachnoid blood clot was surgically removed. In the present case, SSAH occurred during surgery, though the patient did not have any signs of coagulopathy. To the best of our knowledge, this is the first report that describes the occurrence of SSAH during anterior decompression surgery for cervical OPLL. In the present case, the occurrence of SSAH was detected intraoperatively using ultrasonography, and successful removal of the subarachnoid blood clot was performed at an early stage after the SSAH.

Min *et al*[6] analyzed 197 patients who underwent anterior decompression surgery and reported that a dural defect was observed in 25 (12.7%) patients. Similarly, Belanger *et al*[7] reported that 8 (13.1%) of 61 patients with cervical OPLL had absent dura. In the present case, dural ossification was present at the OPLL site at the C3–C4 levels. Because the right C4 root runs near the edge of the OPLL at C3/4, it is possible that we directly injured the radicular artery along the right C4 root when we resected the edge of the OPLL.

The incidence of SSAH has been reported to be low compared with that of spinal epidural hematoma.[8] One reason for the rarity of SSAH is described, in that usually the diluting and redistributing effect of CSF prevents subarachnoid blood from clotting and from forming a solid hematoma.[8] However, when mechanical obstacles are present within the spinal column, such as that occurs in spondylosis, disc herniation, arachnoiditis, or thickening of the yellow ligament, bleeding in the subarachnoid space may result in the formation of an SSAH.[9] In the present case, there was a soft disc herniation at the C5/6 level. We suggest that this is the reason why the SSAH formed at the cranial aspect of the C5/6 level.

Intraoperative ultrasonography has been used during spinal surgery to assess the bony anatomy of the spinal canal as well as the anatomy and pathology of the spinal cord.[10] In the present case, we were able to detect the intrathecal mass intraoperatively by using ultrasonography. On the basis of the findings, we promptly cut the dura and

diagnosed that the mass was a subarachnoid hematoma. If we had not performed the examination, we would have overlooked the SSAH. If we had only noticed the SSAH several days after surgery, severe neural deficits may possibly have developed. From this experience, we believe that intraoperative ultrasonography is a useful tool for detecting intrathecal lesions, including SSAH. Previous studies have shown that, immediately after hemorrhage, focal signal intensity by ultrasonography is high.[11] Thus, when anterior decompression surgery for cervical OPLL is performed, if immediately after a high-intensity mass is detected inside of the dura by ultrasonography, the possible occurrence of a SSAH should be considered.

■ Key Points

- We report a surgically treated case of cervical myelopathy caused by ossification of the posterior longitudinal ligament (OPLL), in which subarachnoid hematoma developed intraoperatively.
- The subarachnoid hematoma was diagnosed on the basis of the findings of intraoperative ultrasonography.
- This experience suggests that, when anterior decompression surgery is performed for cervical OPLL patients, we should consider the possible occurrence of a spinal subarachnoid hematoma.

Acknowledgment

The authors thank Professor Kazuhisa Takahashi (Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine) for his kind support in this work.

References

1. Aghi M, Coumans JV, Brisman JL. Subarachnoid hematoma, hydrocephalus, and aseptic meningitis resulting from a high cervical myelogram. *J Spinal Disord Tech* 2004;17:348–51.
2. Domenicucci M, Ramieri A, Paolini S, et al. Spinal subarachnoid hematomas: our experience and literature review. *Acta Neurochir (Wien)* 2005;147:741–50.
3. Jang WY, Lee JK, Moon KS, et al. Traumatic acute spinal subarachnoid hematoma. *J Clin Neurosci* 2007;14:71–3.
4. Fujiyoshi T, Yamazaki M, Kawabe J, et al. A new concept for making decisions regarding the surgical approach for cervical ossification of the posterior longitudinal ligament: the K-line. *Spine* 2008;33:E990–3.
5. Daentzer D, Deinsberger W, Böker DK. Vertebral artery complications in anterior approaches to the cervical spine: report of two cases and review of literature. *Surg Neurol* 2003;59:300–9.
6. Min JH, Jang JS, Lee SH. Significance of the double-layer and single-layer signs in the ossification of the posterior longitudinal ligament of the cervical spine. *J Neurosurg Spine* 2007;6:309–12.
7. Belanger TA, Roh JS, Hanks SE, et al. Ossification of the posterior longitudinal ligament. Results of anterior cervical decompression and arthrodesis in sixty-one North American patients. *J Bone Joint Surg Am* 2005;87:610–15.
8. Inoue T, Miyamoto K, Kushima Y, et al. Spinal subarachnoid hematoma compressing the conus medullaris and associated with neurofibromatosis type 2. *Spinal Cord* 2003;41:649–52.
9. Komiyama M, Yasui T, Sumimoto T, et al. Spontaneous spinal subarachnoid hematoma of unknown pathogenesis: case reports. *Neurosurgery* 1997;41:691–3.
10. Lammertse D, Dungan D, Dreisbach J, et al. Neuroimaging in traumatic spinal cord injury: an evidence-based review for clinical practice and research. *J Spinal Cord Med* 2007;30:205–14.
11. Mirvis SE, Geisler FH. Intraoperative sonography of cervical spinal cord injury: results in 30 patients. *AJNR Am J Neuroradiol* 1990;11:755–61.

Biochemical and Biophysical Research Communications 377 (2008) 905–909

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A unique mutation of ALK2, G356D, found in a patient with fibrodysplasia ossificans progressiva is a moderately activated BMP type I receptor

Toru Fukuda [a,b], Kazuhiro Kanomata [a], Junya Nojima [a,p], Shoichiro Kokabu [a,p], Masumi Akita [b,c], Kenji Ikebuchi [b,d], Eijiro Jimi [b,e], Tetsuo Komori [b,f], Yuichi Maruki [b,g], Masaru Matsuoka [d], Kohei Miyazono [b,h], Konosuke Nakayama [b,i], Akira Nanba [b,j], Hiroshi Tomoda [b,k], Yasushi Okazaki [b,l], Akira Ohtake [b,m], Hiromi Oda [b,n], Ichiro Owan [b,o], Tetsuya Yoda [b,p], Nobuhiko Haga [q,s], Hirokazu Furuya [r], Takenobu Katagiri [a,b,s,*]

[a] Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Medical University, 1397-1 Yamane, Hidaka-shi, Saitama 350-1241, Japan
[b] Project of Clinical and Basic Research for FOP at Saitama Medical University, 1397-1 Yamane, Hidaka-shi, Saitama 250-1241, Japan
[c] Division of Morphological Science, Biomedical Research Center, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[d] Department of Laboratory Medicine, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[e] Division of Molecular Signaling and Biochemistry, Department of Biosciences, Kyushu Dental Collage, 2-6-1Manazuru, Kokurakita-ku, Kitakyushu-shi, Fukuoka 803-8580, Japan
[f] Department of Neurology, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[g] Department of Neurology, Saitama Neuropsychiatric Institute, 6-11-1 Honchohigashi, Chuo-ku, Saitama-shi, Saitama 338-8577, Japan
[h] Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[i] Department of Endocrinology and Diabetes, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[j] Department of Obstetrics and Gynecology, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[k] Kitasato University School of Pharmaceutical Science, 5-9-1 Shirokane, Minato-ku, Tokyo 108-0023, Japan
[l] Division of Functional Genomics & Systems Medicine, Research Center for Genomic Medicine, Saitama Medical University, 1397-1 Yamane, Hidaka-shi, Saitama 350-1241, Japan
[m] Department of Pediatrics, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[n] Department of Orthopedic Surgery, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[o] Department of Orthopedic Surgery, University of Ryukyus Faculty of Medicine, 207 Uehara, Nishihara-cho, Okinawa 903-0215, Japan
[p] Department of Oral and Maxillofacial Surgery, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[q] Department of Rehabilitation Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
[r] Department of Neurology, Neuro-Muscular Center, National Omuta Hospital, Tachibana 1044-1, Omuta, Fukuoka 837-0911, Japan
[s] The Research Committee on Fibrodysplasia Ossificans Progressiva of the Ministry of Health, Labour and Welfare, Japan

ARTICLE INFO

Article history:
Received 14 October 2008
Available online 24 October 2008

Keywords:
Heterotopic bone formation
Muscle
BMP
Signaling
Receptor

ABSTRACT

Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant congenital disorder characterized by progressive heterotopic bone formation in muscle tissues. A common mutation among FOP patients has been identified in *ALK2*, ALK2(R206H), which encodes a constitutively active bone morphogenetic protein (BMP) receptor. Recently, a unique mutation of ALK2, ALK2(G356D), was identified to be a novel mutation in a Japanese FOP patient who had unique clinical features. Over-expression of ALK2(G356D) induced phosphorylation of Smad1/5/8 and activated Id1-luc and alkaline phosphatase activity in myoblasts. However, the over-expression failed to activate phosphorylation of p38, ERK1/2, and CAGA-luc activity. These ALK2(G356D) activities were weaker than those of ALK2(R206H), and they were suppressed by a specific inhibitor of the BMP-regulated Smad pathway. These findings suggest that ALK2(G356D) induces heterotopic bone formation via activation of a BMP-regulated Smad pathway. The quantitative difference between ALK2(G356D) and ALK2(R206H) activities may have caused the phenotypic differences in these patients.

Fibrodysplasia ossificans progressiva (FOP; OMIM135100) is a rare hereditary disorder with autosomal dominant transmission. Clinical features of FOP are characterized by the presence of malformations of the great toes with hallux valgus and post-natal progressive heterotopic ossification that results in the formation of the ectopic skeleton [1–3]. Bone morphogenetic protein (BMP) signaling has been suggested to be involved in the heterotopic bone formation in FOP patients, since BMPs are capable of inducing ectopic bone formation in muscle and osteoblastic differentiation of myoblasts in vitro [4,5].

Intracellular signaling of BMPs is transduced by two types of serine/threonine kinase receptors: type I and type II [6,7]. Li-

* Corresponding author. Address: Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Medical University, 1397-1 Yamane, Hidaka-shi, Saitama 350-1241, Japan. Fax: +81 42 984 4651.
E-mail address: katagiri@saitama-med.ac.jp (T. Katagiri).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.10.093

gand-bound type II receptor activates type I receptor kinase through phosphorylation of the glycine-serine (GS) domain, which is highly conserved among the type I receptors. Activated BMP type I receptor kinase, in turn, phosphorylates receptor-regulated Smads (R-Smads), which include Smad1, Smad5 and Smad8. Phosphorylated R-Smads form heteromeric complexes with Smad4, a common Smad, and translocate into the nucleus to regulate transcription of various target genes, including Id1[8]. A recurrent heterozygous mutation at 617G>A in the *ACVR1* gene encoding a BMP type I receptor ALK2, was identified in both familial and sporadic patients with FOP [9,10]. This mutation causes an amino acid substitution of Arg to His at codon 206 (R206H) within the GS domain of the ALK2 receptor. We found that ALK2(R206H) is a constitutively activated BMP receptor and cooperatively induces osteoblastic differentiation with Smad1 and Smad5, which are increased during muscle regeneration in vivo [11].

Recently, a unique mutation in the *ALK2* gene, 1067G>A, was identified in a Japanese patient who has slow progressive FOP [12]. This mutation causes an amino acid substitution of Gly to Asp at codon 356 (G356D) in the kinase domain rather than the GS domain of the ALK2 receptor. The clinical pictures of this patient seemed to be unique in comparison with other "classical" FOP patients, since his respiratory problem was slow and progressive, and he had severe hypodactyly in thumbs on both hands, as well as a severe defect in both halluces [12]. In the present study, we found that the ALK2(G356D) mutant receptor activated a Smad-dependent pathway of BMP signaling in the absence of ligands. This method of activation was similar to that for ALK2(R206H) found in the classical FOP patients. These findings suggested that ALK2(G356D) induced heterotopic bone formation by activating BMP signaling as a constitutively activated BMP type I receptor. However, the biological activities of ALK2(G356D) were lower than those of ALK2(R206H). The quantitative difference between ALK2(G356D) and ALK2(R206H) activities may have caused the differences in clinical features in these patients.

Materials and methods

Cell cultures, transfection and reporter assay. C2C12 mouse myoblasts and C3H10T1/2 clone 8 (10T1/2) fibroblasts were maintained in Dulbecco's modified Eagle's medium containing 15% fetal bovine serum [13]. Cells were transfected using the Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. The transcriptional activation induced by ALK2 receptors was measured using IdWT4F-luc or CAGA-luc reporter plasmids, as previously described [8,14]. C2C12 cells were treated with Dorsomorphin (171260, Calbiochem, Darmstadt, Germany) to examine roles of the Smad-dependent signaling on the activities of ALK2(G356D).

Immunoblotting and immunostaining. Cells were lysed in TNE buffer [10 mM Tris–HCl (pH 7.5), 0.15 M NaCl, 1 mM EDTA, and 1% Nonidet P-40] and subjected to immunoblotting, as described previously [11,15]. The following antibodies were used: anti-FLAG antibody (clone M2, Sigma, St. Louis, MO), anti-phosphorylated Smad1/5/8 antibody (Cell Signaling, Beverly, MA), anti-phospho-p38 (SC-7973, SantaCruz, Santa Cruz, CA), anti-phospho-ERK1/2 (SC-7383, SantaCruz) and anti-V5 antibody (Invitrogen). C3H10T1/2 cells were transiently transfected with MyoD to induce myogenesis, which was evaluated by immunohistochemical staining for myosin heavy chain (MHC) using anti-MHC antibody (clone MF-20, Developmental Studies Hybridoma Bank, Iowa City, IA) [16].

Alkaline phosphatase activity. Alkaline phosphatase (ALP) activity was measured as a marker of osteoblast differentiation with a substrate solution (0.1 M diethanolamine, 1 mM $MgCl_2$, and 10 mg/ml *p*-nitrophenylphosphate). Reactions were terminated by adding 3 M NaOH, and the absorbance was measured at 405 nm [13,17].

Statistical analysis. Comparisons were made by using Student's *t*-test. Results were expressed as means ± SD. $P < 0.05$ was considered statistically significant.

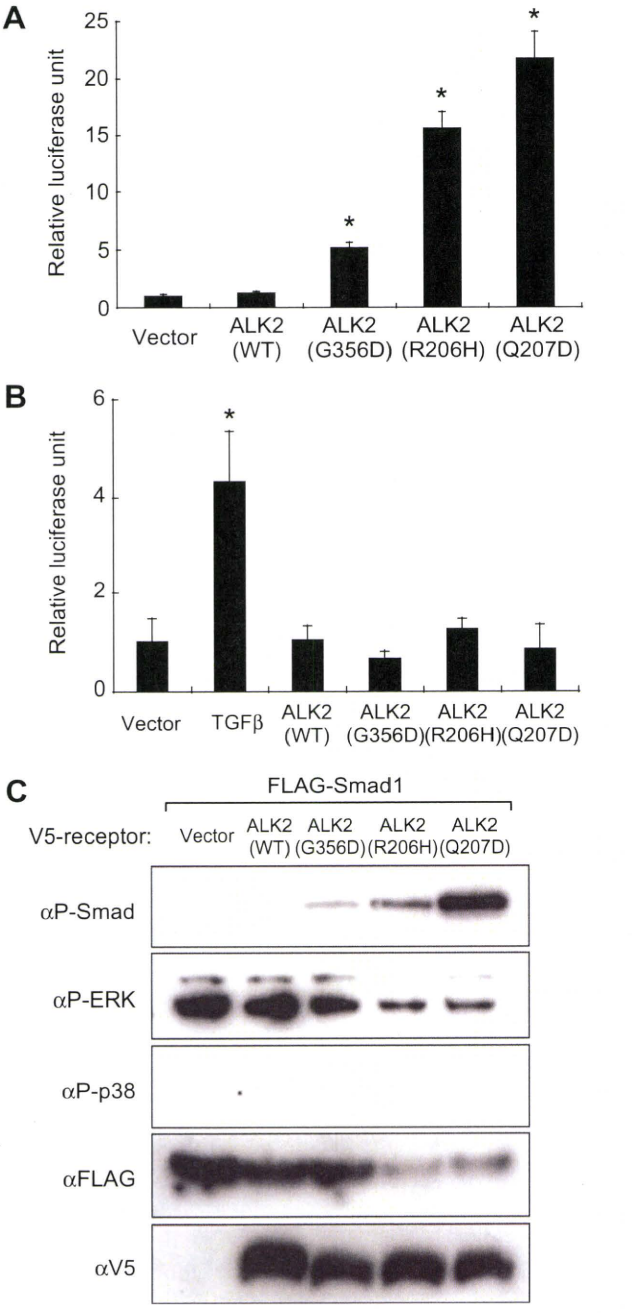

Fig. 1. ALK2(G56D) acts as a constitutively activated BMP receptor. (A,B) Luciferase activities induced by ALK2 receptors. C2C12 cells were co-transfected with IdWT4F-luc (A) or CAGA-luc (B) and with wild-type ALK2, ALK2(G56D), ALK2(R206H), or ALK2(Q207D). Constitutively active ALK2(Q207D) and 5 ng/ml of TGF-β1 were used as positive controls for IdWT4F-luc and CAGA-luc, respectively. Results are represented as means ± SD ($n = 3$). $^{*}P < 0.05$ when compared to vector transfection. (C) Intracellular signaling is activated by ALK2 receptors. C2C12 cells were co-transfected with FLAG-tagged Smad1 and a V5-tagged wild-type ALK2(WT), ALK2(G56D), ALK2(R206H), or ALK2(Q207D). Cell lysates were immunoblotted with anti-phospho-Smad1/5/8, anti-phospho-p38 and anti-phospho ERK antibodies.

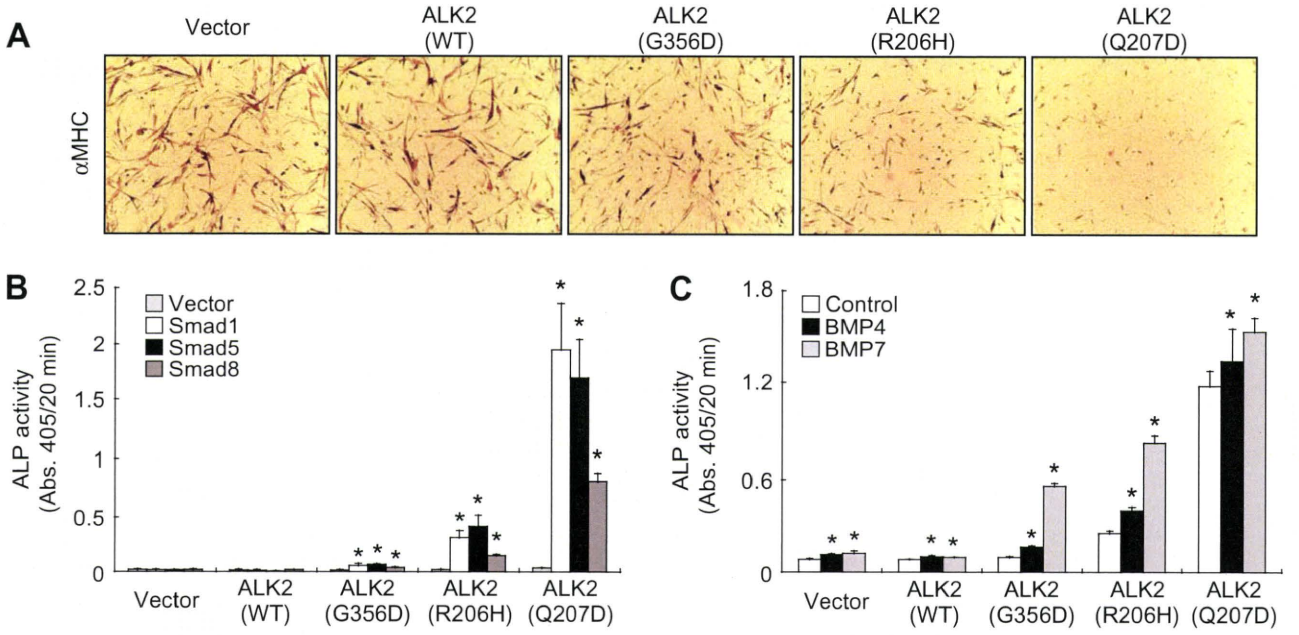

Fig. 2. ALK2(G56D) and Smad1/5 cooperatively induce osteoblastic differentiation. (A) Immunostaining of MHC in C3H10T1/2 cells co-transfected with a MyoD expression construct and an empty vector, wild-type ALK2, ALK2(G56D), ALK2(R206H), or ALK2(Q207D) construct. (B) ALP activities induced by co-operation of ALK2 receptors and Smad1/5/8. C2C12 cells co-transfected with FLAG-tagged Smad1, Smad5, or Smad8 and V5-tagged wild-type ALK2, ALK2(G356D), ALK2(R206H), or ALK2(Q207D). ALP activity was determined on day 3. Results are represented as means ± SD ($n = 3$). $^{*}P < 0.05$ when compared to vector transfection in each group. (C) ALP activities induced by co-operation of ALK2 receptors and BMPs. C2C12 cells co-transfected with Smad1 and wild-type ALK2, ALK2(G356D), ALK2(R206H), or ALK2(Q207D) were treated for 3 days with 100 ng/ml of BMP-4 or 100 ng/ml of BMP-7. ALP activities were determined on day 3. Results are represented as means ± SD ($n = 3$). $^{*}P < 0.05$ when compared with controls.

Results

ALK2(G356D) activates signaling pathway via BMP-regulated Smad in the absence of ligands

First, we asked whether ALK2(G356D) activates intracellular signaling of BMPs in the absence of ligands in a luciferase assay using IdWT4F-luc. ALK2(G356D) increased luciferase activity in C2C12 myoblasts, although the increase in activity was weaker than that induced by ALK2(R206H) and ALK2(Q207D), which had previously been shown to be constitutively active receptors (Fig. 1A). However, none of these ALK2 receptors activated CAGA-luc, a TGF-β/activin reporter (Fig. 1B). The specificity of these mutant ALK2 receptor kinases was further examined in co-transfection with FLAG-Smad1 followed by western blots. All of the mutant receptors induced FLAG-Smad1 phosphorylation in the order of ALK2(G356D) < ALK2(R206H) < ALK2(Q207D), although the receptors did not activate the phosphorylation of p38 or ERK1/2 (Fig. 1C).

ALK2(G356D) inhibits myogenesis and induces osteoblastic differentiation

Next, we examined the effect of ALK2(G356D)-induced signaling on myogenic differentiation. The number of MHC-positive muscle cells induced by transfection of MyoD in 10T1/2 cells was decreased by co-transfecting with one of the ALK2 receptors in an order of wild-type ALK2 < ALK2(G356D) < ALK2(R206H) < ALK2(Q207D) (Fig. 2A).

We recently reported that co-expression of ALK2(R206H) with Smad1, Smad5 or Smad8 cooperatively induced osteoblastic differentiation of C2C12 myoblasts [11]. Co-transfection of ALK2(G356D) with Smad1, Smad5 or Smad8 also significantly induced ALP activity, although the enzyme activities were much lower than those induced by ALK2(R206H) or ALK2(Q207D) (Fig. 2B).

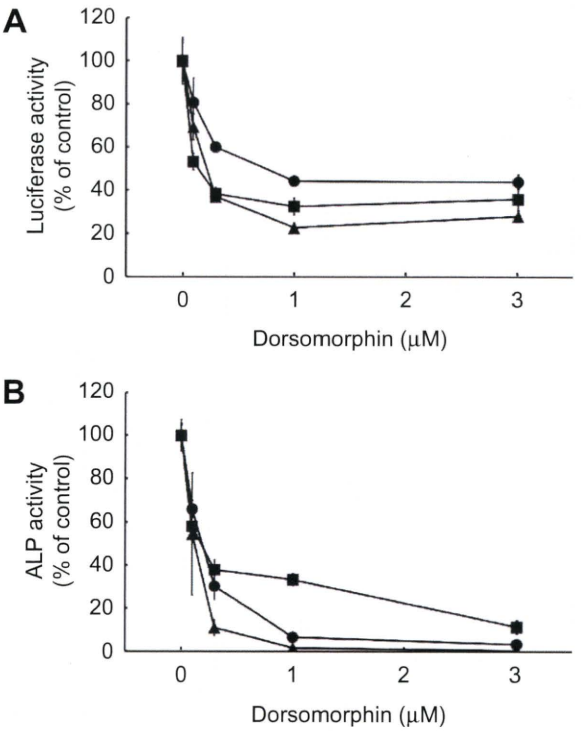

Fig. 3. Dorsomorphin inhibits ALK2(G356D) activity. The biological activities of ALK2(G356D) as determined by IdWT4F-luc (A) and ALP activity (B) were suppressed by Dorsomorphin in C2C12 cells. C2C12 cells were transfected with ALK2(G356D) (closed squares), ALK2(R206H) (closed triangles) or ALK2(Q207D) (closed circles) and treated with graded concentrations of Dorsomorphin. Luciferase activity (A) and ALP activity (B) were determined on day 3. Results are indicated as percentages of control (without Dorsomorphin).

Treatment of C2C12 cells expressing ALK2 receptors with BMP-4 or BMP-7 further increased ALP activity. BMP-7 showed higher activity than BMP-4 in cells transfected with mutant receptors such as ALK2(G356D) (Fig. 2C).

Dorsomorphin inhibited ALK2(G356D) activities in C2C12 myoblasts

Finally, we examined the effect of Dorsomorphin, a specific inhibitor of the Smad-dependent signaling from BMP type I receptors, on the activities of ALK2(G356D) in C2C12 cells [18]. Dorsomorphin inhibited the IdWT4F-luc and ALP activities, as induced by ALK2(G356D), in a dose-dependent manner, suggesting that Dorsomorphin and its derivatives may be useful for treating FOP caused by not only ALK2(R206H) but also ALK2(G356D) (Fig. 3).

Discussion

In the present study, we characterized ALK2(G356D), a unique ALK2 mutant identified in a Japanese patient who had a slow progressive FOP, severe hypodactyly in the thumbs of both hands and a severe defect of both halluces [12]. The G356D mutation is located at the central region of the serine/threonine kinase domain in ALK2 [12]. In contrast, the R206H mutation found in the classical FOP patients is located in the GS domain, which functions as a switch for kinase activity. Given these unique manifestations in the patient, as well as the hypothesis that the rarity of this mutation may be related to the specific activity of ALK2(G356D), we compared the biological activities of ALK2(G356D) and ALK2(R206H) in vitro. Similar to ALK2(R206H), ALK2(G356D) induced Smad1/5/8 phosphorylation and transactivation of *Id1* promoter in C2C12 myoblasts. Moreover, co-transfecting ALK2(G356D) and Smad1/5 into C2C12 cells or treating the C2C12 cells that express ALK2(G356D) with BMPs cooperatively induced osteoblastic differentiation. The effect of ALK2(G356D)-induced osteoblastic differentiation was suppressed by Dorsomorphin, a specific inhibitor of the BMP-regulated Smad pathway. These results supported and further extended our previous findings that the heterotopic bone formation in FOP may be induced by a constitutively active ALK2 through Smad1 or Smad5. Therefore, the inhibition of these mutant ALK2 receptors by specific inhibitors such as Dorsomorphin and its derivatives may represent strategies for blocking heterotopic bone formation in FOP.

Surprisingly, the biological activities of ALK2(G356D) were found to be weaker than those of ALK2(R206H) in spite of the fact that the patient had some severe clinical features. We speculated that ALK2(G356D) may have changed the substrate specificity and activated Smad2/3 pathway in addition to Smad1/5/8. ALK2(G356D) activated IdWT4F-luc, but failed to activate CAGA-luc, suggesting that ALK2(G356D) did not activate the Smad2/3 pathway. In addition, we could not detect any differences between ALK2(G356D) and ALK2(R206H) in other signaling pathways, such as the p38 and ERK1/2 MAP kinase pathways [19]. These results suggested that a quantitative difference in the kinase activities between ALK2(G356D) and ALK2(R206H) on Smad1/5/8 may have caused the difference in clinical features in the FOP patients, although we could not rule out the possibility that other signaling pathways are also involved in these features. A recent report has suggested a novel function for R-Smads in regulating cell functions through DROSHA mediated microRNA maturation [20]. Additional experiments will be required to elucidate these possibilities.

It is interesting to note that the clinical features of the patient who had ALK2(G356D) could be classified into an embryonic developmental phenotype and a post-natal phenotype. The slow, progressive heterotopic bone formation, a typical post-natal phenotype, may be explained by the lower ALK2(G356D) kinase activity compared with the kinase activity in ALK2(R206H). In contrast, the severe hypodactyly in the thumbs of both hands and the severe defect in both halluces in the patient with ALK2(G356D) may be due to patterning defects of digits during embryogenesis. These clinical features in the patient clearly indicated that ALK2 has two distinct types of physiological functions during embryogenesis and during the post-natal period in humans. Establishment of a transgenic mouse carrying the ALK2(G356D) mutation may help elucidate these roles of ALK2 in vertebrates.

In conclusion, ALK2(G356D) is a weakly activated BMP type I receptor that induces activation of the BMP-regulated Smad signaling pathway. Dorsomorphin and its derivatives may represent therapeutic approaches for inhibiting heterotopic bone formation in FOP induced by mutant ALK2 receptors.

Acknowledgments

We thank members of the Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Medical University, for their valuable comments. We are grateful to Dr. J.A. Langer for kindly providing pcDEF3. This work was supported in part by Health and Labour Sciences Research Grants for Research on Measures for Intractable Research from the Ministry of Health, Labour and Welfare of Japan, a grant-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, a grant-in-aid from the Support Project for the Formation of Strategic Research Center in Private University from the Ministry of Education. Culture, Sports, Science and Technology of Japan, a grant-in-aid from the Sankyo Foundation of Life Science, a grant-in-aid from the Kawano Masanori Memorial Foundation for Promotion of Pediatrics, a grant-in-aid from the Novo Nordisk Award for Growth and Development, and a grant in-aid from Japan Intractable Diseases Research Foundation.

References

[1] R.B. Cohen, G.V. Hahn, J.A. Tabas, J. Peeper, C.L. Levitz, A. Sando, N. Sando, M. Zasloff, F.S. Kaplan, The natural history of heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. A study of forty-four patients, J. Bone Joint Surg. Am. 75 (1993) 215–219.
[2] F.S. Kaplan, W. McCluskey, G. Hahn, J.A. Tabas, M. Muenke, M.A. Zasloff, Genetic transmission of fibrodysplasia ossificans progressiva. Report of a family, J. Bone Joint Surg. Am. 75 (1993) 1214–1220.
[3] F.S. Kaplan, J.A. Tabas, F.H. Gannon, G. Finkel, G.V. Hahn, M.A. Zasloff, The histopathology of fibrodysplasia ossificans progressiva. An endochondral process, J. Bone Joint Surg. Am. 75 (1993) 220–230.
[4] M.R. Urist, Bone: formation by autoinduction, Science 150 (1965) 893–899.
[5] J.M. Wozney, V. Rosen, A.J. Celeste, L.M. Mitsock, M.J. Whitters, R.W. Kriz, R.M. Hewick, E.A. Wang, Novel regulators of bone formation: molecular clones and activities, Science 242 (1988) 1528–1534.
[6] K. Miyazono, S. Maeda, T. Imamura, BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk, Cytokine Growth Factor Rev. 16 (2005) 251–263.
[7] T. Katagiri, T. Suda, K. Miyazono, The bone morphogenetic proteins, in: R. Derynck, K. Miyazono (Eds.), The TGF-β Family, Cold Spring Harbor Laboratory Press, New York, 2008, pp. 121–149.
[8] T. Katagiri, M. Imada, T. Yanai, T. Suda, N. Takahashi, R. Kamijo, Identification of a BMP-responsive element in Id1, the gene for inhibition of myogenesis, Genes Cells 7 (2002) 949–960.
[9] E.M. Shore, M. Xu, G.J. Feldman, D.A. Fenstermacher, T.J. Cho, I.H. Choi, J.M. Connor, P. Delai, D.L. Glaser, M. LeMerrer, R. Morhart, J.G. Rogers, R. Smith, J.T. Triffitt, J.A. Urtizberea, M. Zasloff, M.A. Brown, F.S. Kaplan, A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva, Nat. Genet. 38 (2006) 525–527.
[10] M. Nakajima, N. Haga, K. Takikawa, N. Manabe, G. Nishimura, S. Ikegawa, The ACVR1 617G>A mutation is also recurrent in three Japanese patients with fibrodysplasia ossificans progressiva, J. Hum. Genet. 52 (2007) 473–475.
[11] T. Fukuda, M. Kohda, K. Kanomata, J. Nojima, A. Nakamura, J. Kamizono, Y. Noguchi, K. Iwakiri, T. Kondo, J. Kurose, K.I. Endo, T. Awakura, J. Fukushi, Y. Nakashima, T. Chiyonobu, A. Kawara, Y. Nishida, I. Wada, M. Akita, T. Komori, K. Nakayama, A. Nanba, Y. Maruki, T. Yoda, H. Tomoda, P. B. Yu, E.M. Shore, F.S. Kaplan, K. Miyazono, M. Matsuoka, K. Ikebuchi, A. Ohtake, H. Oda, E. Jimi, I. Owan, Y. Okazaki, T. Katagiri, Constitutively activated ALK2 and increased smad1/5 cooperatively induce BMP signaling in fibrodysplasia ossificans progressiva, J. Biol. Chem. (2008), doi:10.1074/jbc.M801681200.

[12] H. Furuya, K. Ikezoe, L. Wang, Y. Ohyagi, K. Motomura, N. Fujii, J. Kira, Y. Fukumaki, A unique case of fibrodysplasia ossificans progressiva with an ACVR1 mutation, G356D, other than the common mutation (R206H), Am. J. Med. Genet. A 146A (2008) 459–463.
[13] T. Katagiri, A. Yamaguchi, M. Komaki, E. Abe, N. Takahashi, T. Ikeda, V. Rosen, J.M. Wozney, A. Fujisawa-Sehara, T. Suda, Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage, J. Cell Biol. 127 (1994) 1755–1766.
[14] H. Suzuki, K. Yagi, M. Kondo, M. Kato, K. Miyazono, K. Miyazawa, c-Ski inhibits the TGF-beta signaling pathway through stabilization of inactive Smad complexes on Smad-binding elements, Oncogene 23 (2004) 5068–5076.
[15] T. Fukuda, K. Kanomata, J. Nojima, I. Urakawa, T. Suzawa, M. Imada, A. Kukita, R. Kamijo, T. Yamashita, T. Katagiri, FGF23 induces expression of two isoforms of NAB2, which are corepressors of Egr-1, Biochem. Biophys. Res. Commun. 353 (2007) 147–151.
[16] T. Katagiri, S. Akiyama, M. Namiki, M. Komaki, A. Yamaguchi, V. Rosen, J.M. Wozney, A. Fujisawa-Sehara, T. Suda, Bone morphogenetic protein-2 inhibits terminal differentiation of myogenic cells by suppressing the transcriptional activity of MyoD and myogenin, Exp. Cell Res. 230 (1997) 342–351.
[17] K. Kodaira, M. Imada, M. Goto, A. Tomoyasu, T. Fukuda, R. Kamijo, T. Suda, K. Higashio, T. Katagiri, Purification and identification of a BMP-like factor from bovine serum, Biochem. Biophys. Res. Commun. 345 (2006) 1224–1231.
[18] P.B. Yu, C.C. Hong, C. Sachidanandan, J.L. Babitt, D.Y. Deng, S.A. Hoyng, H.Y. Lin, K.D. Bloch, R.T. Peterson, Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism, Nat. Chem. Biol. 4 (2008) 33–41.
[19] J.L. Fiori, P.C. Billings, L.S. de la Pena, F.S. Kaplan, E.M. Shore, Dysregulation of the BMP-p38 MAPK signaling pathway in cells from patients with fibrodysplasia ossificans progressiva (FOP), J. Bone Miner. Res. 21 (2006) 902–909.
[20] B.N. Davis, A.C. Hilyard, G. Lagna, A. Hata, SMAD proteins control DROSHA-mediated microRNA maturation, Nature 454 (2008) 56–61.