Biochemical and Biophysical Research Communications 407 (2011) 213–218

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A novel mutation of ALK2, L196P, found in the most benign case of fibrodysplasia ossificans progressiva activates BMP-specific intracellular signaling equivalent to a typical mutation, R206H

Satoshi Ohte [a,b], Masashi Shin [a], Hiroki Sasanuma [a], Katsumi Yoneyama [a], Masumi Akita [b,c], Kenji Ikebuchi [b,d], Eijiro Jimi [b,e], Yuichi Maruki [b,f], Masaru Matsuoka [b,d], Akira Namba [b,g], Hiroshi Tomoda [b,h], Yasushi Okazaki [b,i], Akira Ohtake [b,j], Hiromi Oda [b,k], Ichiro Owan [b,l], Tetsuya Yoda [b,m], Hirokazu Furuya [n], Jyunji Kamizono [o,p], Hiroshi Kitoh [o,q], Yasuharu Nakashima [o,r], Takafumi Susami [o,s], Nobuhiko Haga [o,t], Tetsuo Komori [b,u], Takenobu Katagiri [a,b,o,*]

[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, Japan
[c] Division of Morphological Science, Biomedical Research Center, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[d] Division 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 Neuropsychiatric Institute, 6-11-1 Honchohigashi, Chuo-ku, Saitama-shi, Saitama 338-8577, Japan
[g] Division of Obstetrics and Gynecology, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[h] Graduate School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-0023, Japan
[i] Division of Functional Genomics & Systems Medicine, Research Center for Genomic Medicine, Saitama Medical University, 1397-1 Yamane, Hidaka-shi, Saitama 350-1241, Japan
[j] Division of Pediatrics, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[k] Division of Orthopedic Surgery, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[l] Department of Orthopedic Surgery, University of Ryukyus Faculty of Medicine, 207 Uehara, Nishihara-cho, Okinawa 903-0215, Japan
[m] Division of Oral and Maxillofacial Surgery, Saitama Medical University, 38 Moro Hongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan
[n] Department of Neurology, Neuro-Muscular center, National Omuta Hospital, Tachibana 1044-1, Omuta, Fukuoka 837-0911, Japan
[o] The Research Committee on Fibrodysplasia Ossificans Progressiva of the Ministry of Health, Labour and Welfare, Japan
[p] Kitakyushu City Yahata Hospital Pediatric Emergency Department, 4-18-1 Nishihon-machi, Yahatahigashi-ku, Kitakyushu-shi, Fukuoka 805-0061, Japan
[q] Department of Orthopedic Surgery, Graduate School of Medicine, Nagoya University, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[r] Department of Orthopaedic Surgery, Faculty of Medicine, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka-shi, Fukuoka 812-8582, Japan
[s] Department of Oral-Maxillofacial Surgery, Dentistry and Orthodontics, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
[t] Department of Rehabilitation Medicine, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
[u] Department of Neurology, National Hakone Hospital, 412 Kazamatsuri, Odawara-shi, Kanagawa 250-0032, Japan

ARTICLE INFO

Article history:
Received 28 February 2011
Available online 4 March 2011

Keywords:
FOP
ALK2
BMP
Smad
Muscle
Signaling

ABSTRACT

Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant congenital disorder characterized by progressive heterotopic ossification in muscle tissues. Constitutively activated mutants of a bone morphogenetic protein (BMP) receptor, ALK2, have been identified in patients with FOP. Recently, a novel ALK2 mutation, L196P, was found in the most benign case of FOP reported thus far. In the present study, we examined the biological activities of ALK2(L196P) *in vitro*. Over-expression of ALK2(L196P) induced BMP-specific activities, including the suppression of myogenesis, the induction of alkaline phosphatase activity, increased BMP-specific luciferase reporter activity, and increased phosphorylation of Smad1/5 but not Erk1/2 or p38. The activities of ALK2(L196P) were higher than those of ALK2(G356D), another mutant ALK2 allele found in patients with FOP and were equivalent to those of ALK2(R206H), a typical mutation found in patients with FOP. ALK2(L196P) was equally or more resistant to inhibitors in comparison to ALK2(R206H). These findings suggest that ALK2(L196P) is an activated BMP receptor equivalent to ALK2(R206H) and that ALK2(L196P) activity may be suppressed *in vivo* by a novel molecular mechanism in patients with this mutation.

Abbreviations: ALK2, activin receptor-like kinase 2; ALP, alkaline phosphatase; BMP, bone morphogenetic protein; FOP, fibrodysplasia ossificans progressiva; MAPK, mitogen-activated protein kinase; TGF-β, transforming growth factor-β.

* Corresponding author at: 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 © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.03.001

1. Introduction

Fibrodysplasia ossificans progressiva (FOP; OMIM135100) is a rare hereditary disorder that is characterized by postnatal progressive heterotopic ossification in soft tissues, especially skeletal muscle [1–3]. The heterotopic ossification in FOP starts during childhood, and most patients with FOP shows congenital malformation of the great toes at birth [1–4]. In FOP, acute heterotopic ossification is induced by muscle injury, such as accidental trauma or surgical operations. There is no effective treatment for preventing the heterotopic ossification associated with FOP.

The ACVR1 gene on chromosome 2q23-24 has been identified as the gene responsible for both familial and sporadic cases of FOP [5]. ACVR1 encodes the ALK2 protein, which is one of the signaling receptors for bone morphogenetic proteins (BMPs) [3,6,7]. BMPs induce heterotopic bone formation in skeletal muscle *in vivo* and initiate the differentiation pathway through which myoblasts convert to osteoblastic cells *in vitro* [8,9]. ALK2 is a transmembrane serine/threonine kinase receptor that activates intracellular signaling pathways via Smad1/5, Erk1/2, and p38 in response to BMP binding [3,6,7]. A substitution mutation in ALK2 from arginine to histidine at codon 206 (R206H) has been found in patients with FOP [5]. This ALK2 mutant activates BMP-specific intracellular signaling without binding to ligands, similar to ALK2(Q207), which is an experimentally identified active ALK2 variant [10]. Thus, FOP was the first disease identified as a gain-of-function mutation involving the signaling receptors for BMPs and related ligands.

Several mutations at different positions within ALK2 have been identified in atypical FOP patients who show variations in clinical features, such as the progression of heterotopic ossification and/or finger development [11,12]. Among these mutations, the G356D mutation was found in one patient who had severe toe malformation and mild heterotopic bone formation in muscle in comparison to typical patients with FOP, who have an ALK2(R206H) allele [11]. We reported that ALK2(G356D) was also an activated BMP receptor but that it was weaker than ALK2(R206H) *in vitro* [13]. It has been suggested that quantitative differences in the biological activities of ALK2 mutants may cause the differences in clinical features in patients with FOP [13]. However, the molecular mechanisms of the regulation of these mutant ALK2 receptors are still unclear.

Recently, a novel mutation in ALK2, L196P, was found in the most benign FOP variant case reported in the literature thus far [14]. In contrast to other patients with FOP, this patient had neither great toe malformations at birth nor heterotopic ossification in skeletal muscle until a motorcycle accident at age 21 [14]. To examine the molecular mechanisms of this mild phenotype, we compared the biological activities of ALK2(L196P) with other ALK2 mutants *in vitro*. Unexpectedly, we found that ALK2(L196P) showed almost equivalent activities compared to ALK2(R206H), and it was less sensitive than ALK2(R206H) to protein inhibitors, such as Smad6 and FKBP12. These findings suggest that the activity of L196P might be masked by a novel *in vivo* mechanism in patients with this mutation. Such an inhibitory mechanism may aid in the establishment of a new treatment strategy to prevent heterotopic ossification in FOP.

2. Material and methods

2.1. Plasmids and cell cultures

The V5-tagged human ALK2 expression vector was used [12,13]. ALK2(L196P) was obtained via a standard PCR technique using the primers CAGGAAGTGGCTCTGGTCCTCCTTTTCTGGTAC and GTACCAGAAAAGGAGGACCAGAGCCACTTCCTG, and its DNA sequence was confirmed using an ABI3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). A new version of the BMP-specific IdWT4F-luc reporter was constructed by transferring four copies of the BMP-responsive element from the original IdWT4F-luc in pGL3-Promoter [15] to pGL4.26 (Promega, Madison, WI). Other ALK2 mutants, CAGA-luc, and FLAG-tagged Smad1, Smad5, Smad6, Smad7, Smad8, FKBP12, and MyoD have been described previously [12,13,15,16]. Murine C2C12 myoblasts and C3H10T1/2 clone 8 fibroblasts were maintained in Dulbecco's modified Eagle's medium containing 15% fetal bovine serum [9,17].

2.2. Luciferase reporter assay

The luciferase reporter assay was performed using IdWT4F-luc or CAGA-luc with phRL-SV40 for normalization of transfection efficiency [15,18]. C2C12 cells were inoculated at 1×10^4 cells/well in a 96-well plate one day prior to the assay. The cells were transfected with 200 ng of plasmid DNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The firefly and renilla luciferase activities were determined using the Dual-Glo Luciferase Assay Kit (Promega).

2.3. Alkaline phosphatase activity

Alkaline phosphatase (ALP) activity was determined as a typical marker of osteoblastic differentiation [9,19]. C2C12 cells were incubated in a substrate solution (0.1 M diethanolamine, 1 mM $MgCl_2$, and 1 mg/ml p-nitrophenylphosphate) for 30 min at room temperature. Reactions were terminated by adding 3 M NaOH, and the absorbance was measured at 405 nm [19].

2.4. Immunostaining

Myogenesis was induced in C3H10T1/2 cells and tested using immunohistochemical staining of myosin heavy chain (MHC) on day 3 using an antibody (clone MF-20, Developmental Studies Hybridoma Bank, Iowa City, IA) and a Histofine SimpleStain Kit (Nichirei, Tokyo, Japan) [9,17]. The cellular localization of phospho-Smad1/5 and V5-tagged ALK2 in C2C12 cells was determined by immunohistochemical staining using an anti-phospho-Smad1/5 rabbit monoclonal antibody (clone 41D10, Cell Signaling, Beverly, MA) and an anti-V5 mouse monoclonal antibody (clone V5005, Nacalai Tesque, Kyoto, Japan), respectively [10,13,16]. Target proteins were visualized using an Alexa Fluor 488- or Alexa Fluor 594-conjugated secondary antibody (Invitrogen).

2.5. Western blot analysis

C2C12 cells were lysed in TNE buffer and subjected to western blotting as described previously [10,13,16]. The following antibodies were used: anti-phospho-Smad1/5/8 rabbit polyclonal (Cell Signaling), anti-phospho-p38 rabbit monoclonal (clone 3D7, Cell Signaling), anti-phospho-Erk1/2 rabbit monoclonal (clone D13.14.4E, Cell Signaling), anti-FLAG mouse monoclonal (clone M2, Sigma, St. Louis, MO), anti-V5 mouse monoclonal and anti-α-tubulin rabbit polyclonal (Cell Signaling). The target proteins were detected using a horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG antibody (GE Healthcare, Buckinghamshire, England) and a Chemi-Lumi One Super (Nacalai Tesque).

2.6. Statistical analysis

Comparisons were made using Student's *t*-test. Results were expressed as the mean ± SD ($n = 3$). Statistical significance was indicated as $^{*}p < 0.05$ and $^{**}p < 0.01$.

3. Results

3.1. ALK2(L196P) is an activated BMP receptor equivalent to ALK2(R206H)

First, we compared the biological activities of ALK2(L196P) with other ALK2 mutants found in FOP: R206H, G356D, and an experimentally established active mutant, Q207D. In the absence of BMPs, the BMP-specific luciferase reporter IdWT4F-luc was activated 30-fold, 37-fold, 13-fold and 46-fold by the ALK2 mutants L196P, R206H, G356D and Q207D, respectively (Fig. 1A). In contrast to IdWT4F-luc, a TGF-β/activin-specific reporter, CAGA-luc, was not activated by any ALK2 receptor examined (Fig. 1B). The *in vitro* suppression of myogenesis was examined as another marker of BMP activity. The numbers of myosin heavy chain-expressing myogenic cells were decreased in response to the over-expression of ALK2 mutants but not wild-type ALK2 (Fig. 1C). Treatment of parental C2C12 cells with 50 ng/ml of BMP-6 increased alkaline phosphatase activity, which was further increased in response to the over-expression of ALK2 mutant L196P, R206H, G356D, and Q207D alleles (Fig. 1D). In the absence of BMP-6, weak ALP activity was induced only in response to ALK2(Q207D). These findings indicate that ALK2(L196P) is an activated BMP receptor equivalent to ALK2(R206H).

3.2. ALK2(L196P) activates osteoblastic differentiation via the BMP-specific Smad-dependent pathway

We next examined intracellular signaling pathways involved in the BMP activities induced by ALK2(L196P). Over-expression of ALK2(L196P), ALK2(R206H) or ALK2(Q207D) induced phosphorylation of endogenous Smad1/5 in the nuclei of C2C12 cells (Fig. 2A). These mutant ALK2 proteins induced phosphorylation of exogenous FLAG-Smad1 in the following order: Q207D ≫ L196P = R206H ≫ G356D. Neither phospho-Erk1/2 nor phospho-p38 was induced by any mutant ALK2 receptor (Fig. 2B). Co-transfection of one of the mutant ALK2 receptors into C2C12 cells increased ALP activity in cooperation with not only FLAG-Smad1 but also FLAG-Smad5 and FLAG-Smad8 (Fig. 2C). As reported previously, Smad8 showed the lowest activity [10]. The order of ALP activity induction was similar to that of the capacity to phosphorylate exogenous FLAG-Smad1, suggesting that the BMP-specific Smad pathway mediates ALK2-induced osteoblastic differentiation. This result was confirmed by the suppression of ALP activity induced by ALK2 and FLAG-Smad1 by LDN-193189, a specific chemical inhibitor of the phosphorylation of Smad1/5/8 by BMP receptors (Fig. 2D).

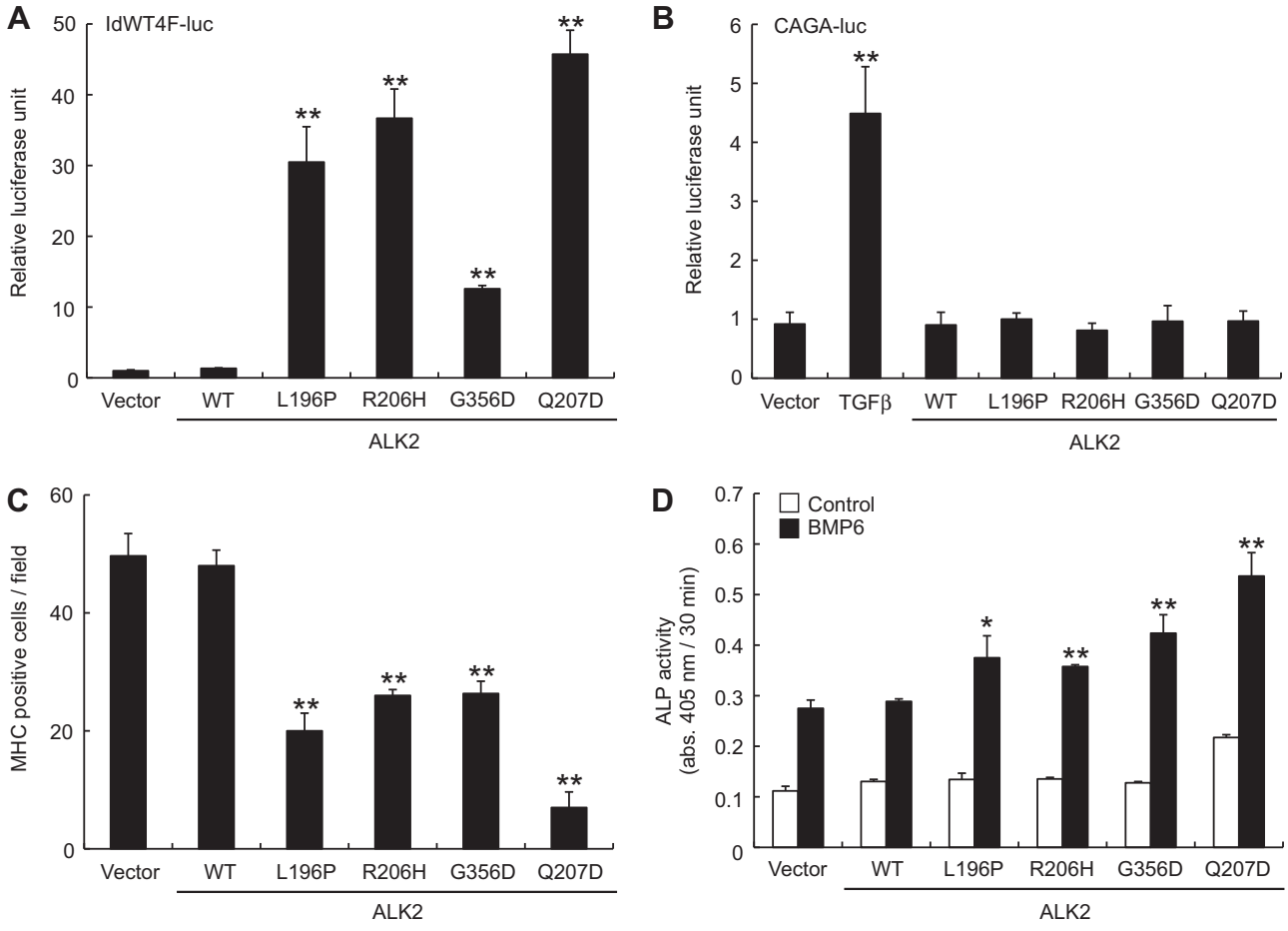

Fig. 1. ALK2(L196P) is an activated BMP receptor. (A and B) BMP-specific and TGF-β-specific luciferase assay. IdWT4F-luc (A) or CAGA-luc (B) was co-transfected with wild-type (WT) or one mutant allele of ALK2 (L196P, R206H, G356D or Q207D) into C2C12 cells. (C) Suppression of myogenesis by ALK2. The numbers of myosin heavy chain (MHC)-positive cells were counted on day 3 in C3H10T1/2 cell cultures transfected with MyoD and one of the ALK2 plasmids as indicated. (D) ALP activity induced by the cooperation of ALK2 and BMP-6. C2C12 cells were transfected with one of the ALK2 plasmids as indicated and cultured for three days with or without 50 ng/ml of BMP-6. Results are presented as the means ± SD ($n = 3$). $^{*}P < 0.05$ and $^{**}P < 0.01$ in comparison with cells transfected with an empty vector in each group.

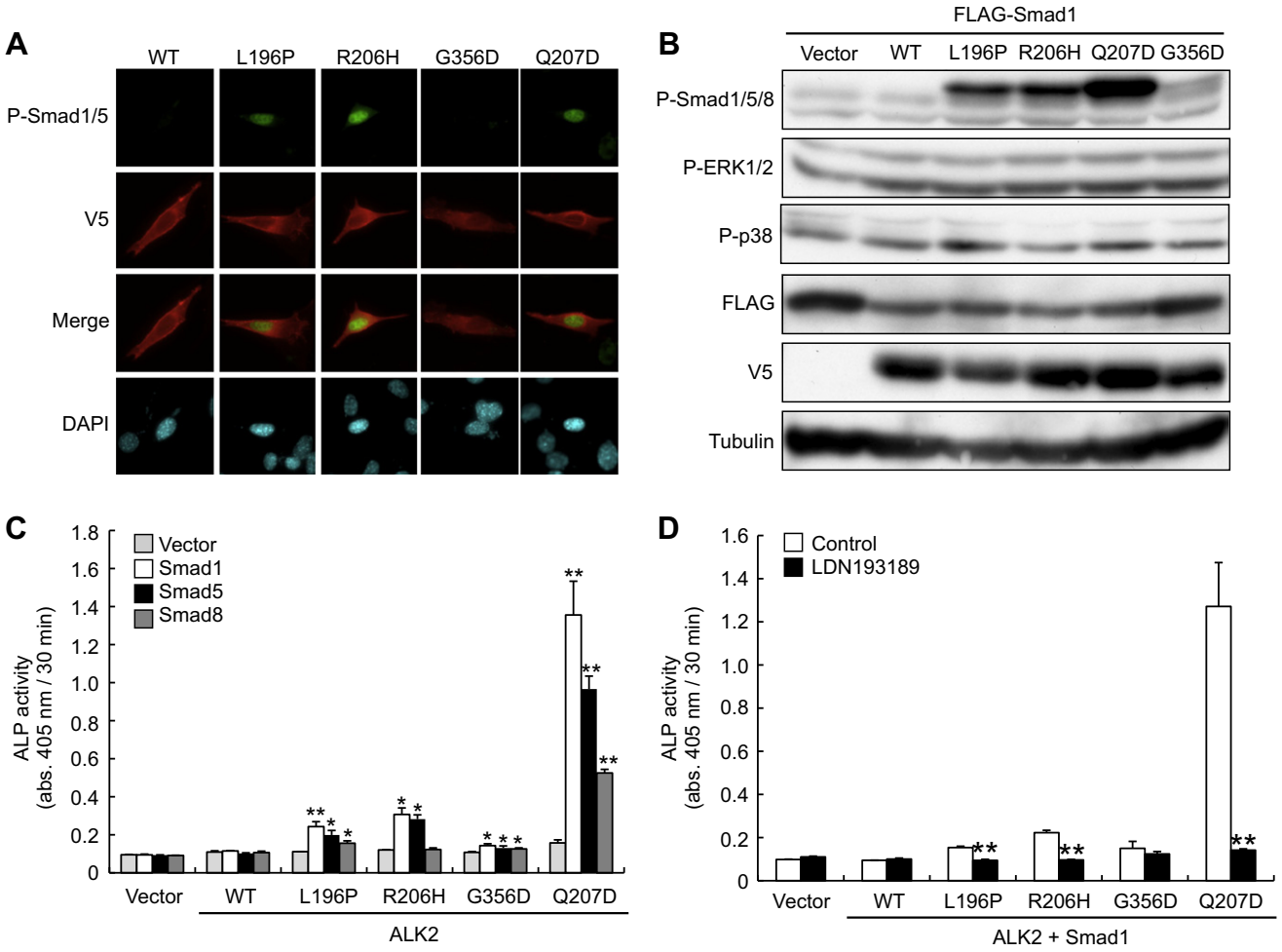

Fig. 2. ALK2(L196P) induces the Smad1/5-dependent pathway but not the non-Smad- or the Smad2/3-dependent pathways. (A) Immunohistochemical analysis of endogenous phospho-Smad1/5. C2C12 cells transfected with one of the V5-tagged ALK2 plasmids indicated were double-stained with anti-phospho-Smad1/5 (green) and anti-V5 (red) antibodies. (B) Western blot analysis of intracellular signaling pathways activated by ALK2 receptors. C2C12 cells were co-transfected with FLAG-tagged Smad1 and one of the V5-tagged ALK2 constructs. Cell lysates were immunoblotted with antibodies against phospho-Smad1/5/8, phospho-Erk1/2, phospho-p38, FLAG-tag, V5-tag and a-tubulin. (C) ALP activity induced by the cooperation of ALK2 and Smad1/5/8. C2C12 cells were co-transfected with one of the ALK2 plasmids as indicated and FLAG-tagged Smad1, Smad5 or Smad8 and cultured for three days in the absence of BMPs. The results are presented as the means ± SD ($n = 3$). $^{*}P < 0.05$ and $^{**}P < 0.01$ in comparison with cells transfected with an empty vector in each group. (D) Activation of the BMP-regulated Smads is involved in osteoblastic differentiation induced by ALK2. C2C12 cells were co-transfected with one of the ALK2 plasmids as indicated and FLAG-tagged Smad1 and cultured for three days with or without 100 nM LDN-193189. Results are presented as the means ± SD ($n = 3$). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with controls.

3.3. Effects of protein and chemical inhibitors on mutant ALK2 activities

To examine the molecular mechanisms of the mild phenotypes of the FOP patient with activated ALK2(L196P), we examined the sensitivity of ALK2(L196P) to protein inhibitors and compared it to that of ALK2(R206H). Co-expression of Smad6, Smad7 or FKBP-12 dose-dependently suppressed the IdWT4F-luc activity induced by ALK2(L196P) or ALK2(R206H). The IC_{50} values of inhibitors against ALK2(L196P) were higher than those of ALK2(R206H) (Fig. 3A–C). The IdWT4F-luc induced by ALK2(L196P) or ALK2(R206H) was dose-dependently suppressed by the chemical inhibitor LDN-193189 (Fig. 3D). However, ALK2(L196P) was slightly more resistant than ALK2(R206H) to LDN-193189 (Fig. 3D).

4. Discussion

In the present study, we examined the molecular mechanisms of a novel ALK2 mutation, ALK2(L196P), found in the most benign case of FOP reported in the literature thus far [14]. We speculated that ALK2(L196P) was a more weakly activating mutation than other ALK2 mutations found in typical and atypical FOP patients because the patient with this mutation did not have toe malformations at birth and had delayed induction of heterotopic ossification in skeletal muscle [14]. Unexpectedly, however, ALK2(L196P) showed higher activity than ALK2(G356D) and equivalent activity to ALK2(R206H), a typical FOP variant mutation, suggesting that ALK2(L196P) activity may be suppressed by a novel mechanism in this patient. To examine this possibility, we compared the sensitivity of ALK2(L196P) receptors to different protein inhibitors, including FKBP12, that have been shown to be involved in the activation of ALK2 in FOP [20]. However, ALK2(L196P) was more resistant rather than more sensitive to Smad6 and FKBP12 than was ALK2(R206H). Thus, a novel inhibitor may be involved in the regulation of ALK2(L196P) *in vivo*. It is possible that such an inhibitory mechanism of ALK2 activity will aid in the establishment of novel therapeutic strategies for FOP patients with other ALK2 mutations. Further studies will be required to examine this possibility.

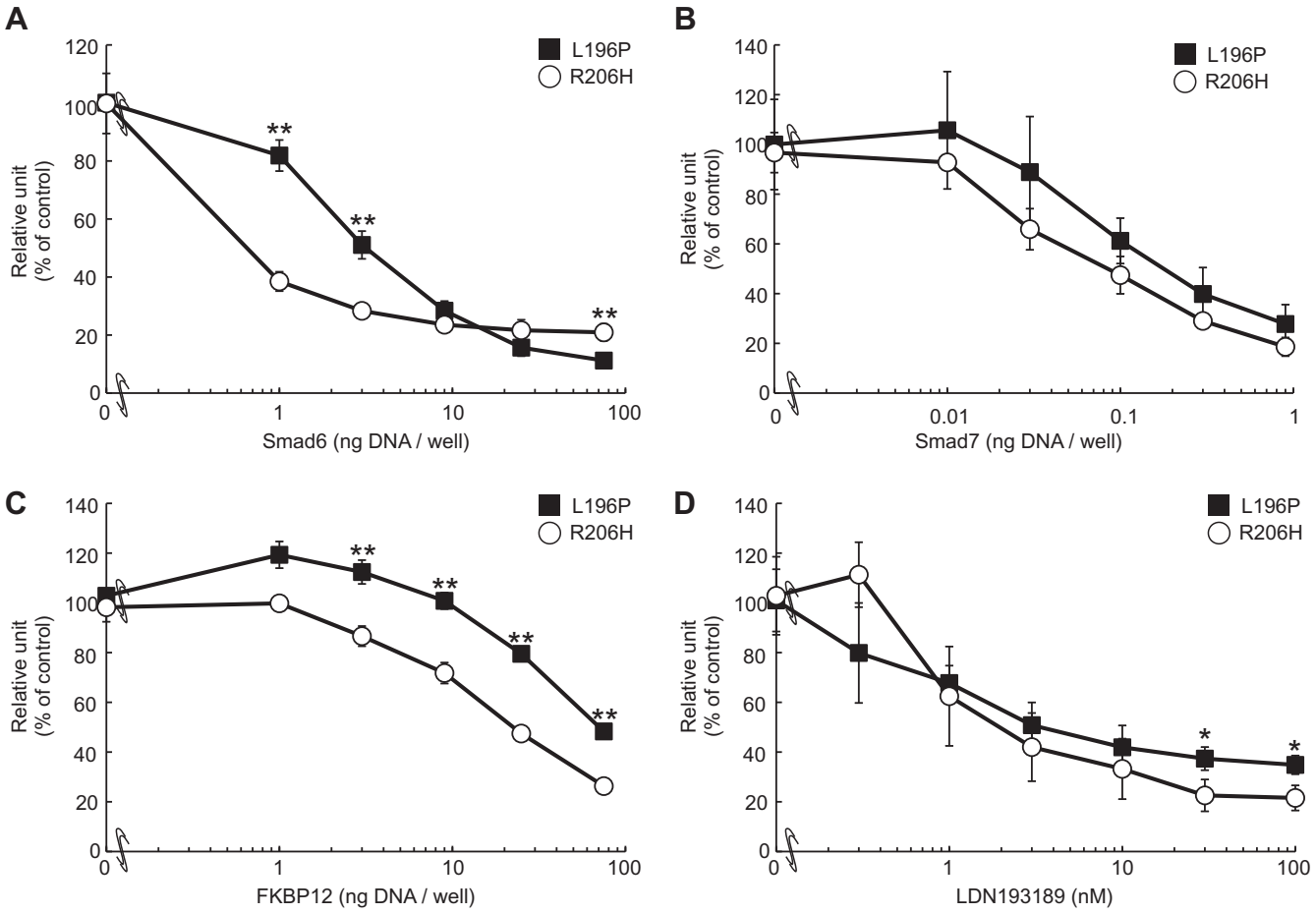

Fig. 3. Effects of protein and chemical inhibitors on ALK2(L196P) and ALK2(R206H). The BMP-specific IdWT4F-luc was activated by the transfection of C2C12 cells with ALK2(L196P) (closed squares) or ALK2(R206H) (open circles). The effects of inhibitors (Smad6 (A), Smad7 (B), FKBP12 (C) and LDN-193189 (D)) on ALK2(L196P) and ALK2(R206H) were determined using IdWT4F-luc in C2C12 cells. The cells were co-transfected with IdWT4F-luc, one of the ALK2 plasmids as indicated and varying amounts of Smad6 (A), Smad7 (B), or FKBP12 (C). The cells were cultured in the presence of varying concentrations of LDN-193189 (D). Total amounts of transfected DNA were adjusted equally with an empty vector. Luciferase activity was determined on day 1. Results are presented as the means (% of control) ± SD ($n = 3$). $^{*}P < 0.05$ and $^{**}P < 0.01$ in comparison with transfection with L196P and R206H.

The induction of the osteoblastic differentiation of myoblasts by ALK2 seems to be dependent on its kinase activity, which phosphorylates Smad1/5 at its carboxyl termini [13]. The degree to which ALP activity was induced by the co-transfection of ALK2 and Smad1 was the same as the degree of the phosphorylation levels of Smads by ALK2 mutants. Moreover, ALK2(L196P) and other mutant ALK2 variants did not activate the non-Smad MAPK or the TGF-b-specific Smad pathways. Recently, we showed that a constitutively activated Smad1, in which the carboxyl phosphorylation sites of BMP receptors were substituted with different acidic amino acids, induced osteoblastic differentiation of C2C12 myoblasts in the absence of BMPs [16]. Taken together, these findings suggest that the phosphorylation of Smad1/5 by mutant ALK2 receptors is one of the most critical targets to prevent heterotopic ossification in FOP. Indeed, the osteoblastic differentiation of C2C12 cells induced by ALK2 mutants was blocked by the chemical inhibitors dorsomorphin and LDN-193189 [10,13]. LDN-193189 has been shown to suppress heterotopic ossification induced by ALK2(Q207D) *in vivo* [21].

In conclusion, a mutant ALK2, ALK2(L196P), found in the most benign case of FOP reported thus far is an activated BMP receptor in the absence of BMPs, and it has equivalent *in vitro* activity to a typical mutation, ALK2(R206H). The *in vivo* biological activity of ALK2(L196P) may be masked by a novel, as yet undiscovered molecular mechanism in this patient.

Acknowledgments

We thank Dr. Kohei Miyazono, Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo and 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, grants-in-aid from The Ministry of Education, Culture, Sports, Science, and Technology of Japan, a grant-in-aid from The Takeda Science Foundation, a grant-in-aid from The Suzuken memorial foundation, and a grant-in-aid for the "Support Project of Strategic Research Center in Private Universities" from The Ministry of Education, Culture, Sports, Science and Technology (MEXT) to Saitama Medical University Research Center for Genomic Medicine.

References

[1] F.S. Kaplan, Q. Shen, V. Lounev, P. Seemann, J. Groppe, T. Katagiri, R.J. Pignolo, E.M. Shore, Skeletal metamorphosis in fibrodysplasia ossificans progressiva (FOP), J. Bone Miner. Metab. 26 (2008) 521–530.

[2] F.S. Kaplan, M. Le Merrer, D.L. Glaser, R.J. Pignolo, R.E. Goldsby, J.A. Kitterman, J. Groppe, E.M. Shore, Fibrodysplasia ossificans progressiva, Best Pract. Res. Clin. Rheumatol. 22 (2008) 191–205.

[3] T. Katagiri, Heterotopic bone formation induced by bone morphogenetic protein signaling: fibrodysplasia ossificans progressiva, J. Oral Biosci. 52 (2010) 33–41.
[4] K. Mishima, H. Kitoh, T. Katagiri, H. Kaneko and N. Ishiguro, Early clinical and radiological characteristics in fibrodysplasia ossificans progressiva. A report of two cases, J. Bone Joint Surg. (2011), in press.
[5] 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.
[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 Suda, T.K. Miyazono, The bone morphogenetic proteins, In The TGF-b Family, in: R. Derynck, K. Miyazono (Eds.), Cold Spring Harbor Press, New York, 2008, pp. 121–149.
[8] M.R. Urist, Bone: formation by autoinduction, Science 150 (1965) 893–899.
[9] 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.
[10] 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, O. Akira, O. Hiromi, J. Eijiro, O. Ichiro, O. Yasushi, K. Takenobu, Constitutively activated ALK2 and increased smad1/5 cooperatively induce BMP signaling in fibrodysplasia ossificans progressiva, J. Biol. Chem. 284 (2009) 7149–7156.
[11] 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.
[12] F.S. Kaplan, M. Xu, P. Seemann, J.M. Connor, D.L. Glaser, L. Carroll, P. Delai, E. Fastnacht-Urban, S.J. Forman, G. Gillessen-Kaesbach, J. Hoover-Fong, B. Koster, R.M. Pauli, W. Reardon, S.A. Zaidi, M. Zasloff, R. Morhart, S. Mundlos, J. Groppe, E.M. Shore, Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1, Hum. Mutat. 30 (2009) 379–390.
[13] T. Fukuda, K. Kanomata, J. Nojima, S. Kokabu, M. Akita, K. Ikebuchi, E. Jimi, T. Komori, Y. Maruki, M. Matsuoka, K. Miyazono, K. Nakayama, A. Nanba, H. Tomoda, Y. Okazaki, A. Ohtake, H. Oda, I. Owan, T. Yoda, N. Haga, H. Furuya, T. Katagiri, A unique mutation of ALK2, G356D, found in a patient with fibrodysplasia ossificans progressiva is a moderately activated BMP type I receptor, Biochem. Biophys. Res. Commun. 377 (2008) 905–909.
[14] C.L. Gregson, P. Hollingworth, M. Williams, K.A. Petrie, A.N. Bullock, M.A. Brown, J.H. Tobias, J.T. Triffitt, A novel ACVR1 mutation in the glycine/serine-rich domain found in the most benign case of a fibrodysplasia ossificans progressiva variant reported to date, Bone 48 (2011) 654–658.
[15] 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.
[16] J. Nojima, K. Kanomata, Y. Takada, T. Fukuda, S. Kokabu, S. Ohte, T. Takada, T. Tsukui, T.S. Yamamoto, H. Sasanuma, K. Yoneyama, N. Ueno, Y. Okazaki, R. Kamijo, T. Yoda, T. Katagiri, Dual roles of smad proteins in the conversion from myoblasts to osteoblastic cells by bone morphogenetic proteins, J. Biol. Chem. 285 (2010) 15577–15586.
[17] 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.
[18] 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.
[19] 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.
[20] Q. Shen, S.C. Little, M. Xu, J. Haupt, C. Ast, T. Katagiri, S. Mundlos, P. Seemann, F.S. Kaplan, M.C. Mullins, E.M. Shore, The fibrodysplasia ossificans progressiva R206H ACVR1 mutation activates BMP-independent chondrogenesis and zebrafish embryo ventralization, J. Clin. Invest. 119 (2009) 3462–3472.
[21] P.B. Yu, D.Y. Deng, C.S. Lai, C.C. Hong, G.D. Cuny, M.L. Bouxsein, D.W. Hong, P.M. McManus, T. Katagiri, C. Sachidanandan, N. Kamiya, T. Fukuda, Y. Mishina, R.T. Peterson, K.D. Bloch, BMP type I receptor inhibition reduces heterotopic ossification, Nat. Med. 14 (2008) 1363–1369.