Histol Histopathol (2013) 28: 473-480
DOI: 10.14670/HH-28.473

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Expression of receptor for hyaluronan-mediated motility (RHAMM) in ossifying fibromas

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Summary. Fibro-osseous lesions of the jaw are poorly understood because of a significant overlap of clinical, radiological and histological features among the various types, though they present distinct patterns of disease progression. An ossifying fibroma is associated with significant cosmetic and functional disturbances, as it shows expansive proliferation. Thus, it is important to establish a specific marker, as well as clearly elucidate its etiology for diagnosis and proper treatment.

We previously established immortalized cell lines from human ossifying fibromas of the jaw and found that they highly expressed the receptor for hyaluronan (HA)-mediated motility (RHAMM). In this study, we examined the expression of RHAMM mRNA in 65 fibro-osseous lesions, including ossifying fibroma, fibrous dysplasia and osseous dysplasia, as well as 5 normal jaws, using real-time RT-PCR and immunohistochemistry assays. RHAMM mRNA and protein expression were significantly elevated in the ossifying fibroma specimens.

These results suggest that detection of upregulated RHAMM expression in an ossifying fibroma assists with differential diagnosis and has a key role in elucidation of its pathophysiology.

Key words: Fibro-osseous lesion, Ossifying fibroma, RHAMM

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Introduction

An ossifying fibroma, previously called cementifying fibroma or cemento-ossifying fibroma, is a member of fibro-osseous lesions of the jaw, which are characterized by replacement of bone with cellular fibrous tissue containing mineralization foci that vary in amount and appearance. An ossifying fibroma is associated with significant cosmetic and functional disturbances, as it is a true benign neoplasm that shows expansive proliferation. During resection, this tumor should be completely enucleated from surrounding bone because of the risk for recurrence, indicating the importance of distinction from other non-neoplastic fibro-osseous lesions. However, accurate diagnosis is difficult because the etiology has not been clarified and no specific marker has been reported. Differential diagnosis based only on pathology findings is difficult because of similar histological features, thus clinical and radiological features are also referenced. Many groups have attempted to clarify the classification of these tumors (Waldron, 1993; Slootweg, 1996; Brannon and Fowler, 2001; Paul and Roman, 2006) and a concept was proposed in the latest WHO classification (Barnes et al., 2005). However, fibro-osseous lesions make up a diverse collection of disorders that include neoplastic and non-neoplastic diseases, while a number of other non-fibro-osseous lesions exhibit findings that may closely mimic those seen in fibro-osseous lesions.

For example, it can be difficult to distinguish focal osseous dysplasia from a conventional ossifying fibroma. The distinguishing features between these 2 types of lesions were exhaustively reviewed by Su et al., in 1997, which showed that osseous dysplasia may be a developmental or possibly hamartomatous condition. A key feature of ossifying fibroma that particularly distinguishes it from fibrous dysplasia is the pattern of mineralization that varies within the lesion, whereas that

in fibrous dysplasia tends to be uniform throughout, though it may be indistinguishable from osseous dysplasia. On the other hand, an ossifying fibroma in many respects may be regarded as a type of osteoblastoma and, in some cases, histological distinction from osteoblastoma may be difficult (Paul and Roman, 2006). In addition, an ossifying fibroma may also be confused with a cementoblastoma, which is an odontogenic neoplasm thought to represent a true tumor composed of cementum. In fragmented biopsies a cementoblastoma may be confused with all types of fibro-osseous lesions, though it generally contains more plump osteoblasts and is radiographically presented as a well-demarcated radiopacity attached to the apex of a tooth, usually a lower molar. Furthermore, an osteoma is a solitary benign neoplasm that may resemble other fibro-osseous lesions (Gitelis and Schajowicz, 1989; Cerase and Priolo, 1998).

We previously established immortalized cell lines from a human ossifying fibroma of the jaw (Kudo et al., 2002) and found that the receptor for hyaluronan (HA)-mediated motility (RHAMM) was highly expressed in comparison with normal osteoblasts obtained from normal human mandibular bone in microarray analysis findings (Hatano et al., 2011, 2012). RHAMM was first described as a soluble hyaluronan binding protein released by sub-confluent migrating cells (Turley, 1982), and its overexpression has been reported in different tumor types, including cases of multiple myeloma, breast cancer, endometrial cancer and colorectal cancer (Wang et al., 1998; Crainie et al., 1999; Rein et al., 2003; Yamano et al., 2008; Lugli et al., 2006). Human RHAMM cDNA was cloned and found to contain 725 amino acids encoding an 84-kDa protein (Wang et al., 1996). Three distinct RHAMM gene products, full-length RHAMM, a splice variant with a 48-bp deletion ($RHAMM^{-48}$) and a variant with a 147-bp deletion ($RHAMM^{-147}$), were cloned from multiple myeloma cells (Crainie et al., 1999), and all of the detected RHAMM splice variants were found to contain exon 4, which is alternatively spliced in murine RHAMM (Crainie et al., 1999).

In the present study, we examined specimens obtained from cases of fibro-osseous lesions to detect the characteristic features of ossifying fibroma. We investigated the expression of RHAMM to clarify the correlation between that expression and clinico-pathological factors, and also examined the expressions of RHAMM splice variants ($RHAMM^{-48}$ and $RHAMM^{-147}$). Furthermore, to clarify the correlation of RHAMM in ossifying fibromas, we examined expressions of the osteogenic markers, alkaline phosphatase (ALP), bone sialoprotein (BSP), and osteocalcin (OCN).

Materials and methods

Experimental subjects

The records of 65 patients with fibro-osseous lesions treated during the past 25 years at our department were obtained. Radiographs and clinical data showing demographic information, location and size of the mass, duration of symptoms, type of biopsy, initial diagnosis, type of operative treatment and complications for all patients were obtained. In addition, we obtained formalin-fixed and paraffin-embedded (FFPE) tissue blocks from the histopathology files of all 65 cases, as well as from 5 normal mature jaws with no fibro-osseous lesions for use in this study. All specimens were obtained after receiving informed consent from the subjects and approval from the Institutional Review Board of Hiroshima University Hospital.

RT-PCR analysis

Total RNA was extracted from FFPE tissue blocks using an RNeasy® FFPE Kit (QIAGEN), then 50 ng of total RNA was subjected to RT-PCR using a first-strand cDNA synthesis kit (Amersham Biosciences). mRNA levels were quantified using a real-time fluorescence detection method (Kubista et al., 2006). Fluorescence was detected using a fluorescent quantitative detection system equipped with a paser detector (LineGene FQD-33A, Bio Flux) by measuring the binding of the fluorescent dye SYBR-Green I to double-stranded DNA. PCR assays were performed in microtubes at a volume of 20 μl with a reaction mixture containing 1.0 μg of cDNA, 10 μl of SYBR-Green PCR master mix (Toyobo) and 10 pmol of each pair of oligonucleotide primers. The oligonucleotide RT-PCR primers were purchased from Hokkaido System Science and are listed in Table 1. The PCR program was as follows: initial melting at 95°C for 30 seconds, followed by 40 cycles at 95°C for 15 seconds, 58°C for 10 seconds and 72°C for 15 seconds. To confirm amplification specificity, the PCR products were subjected to subsequent agarose gel electrophoresis. The threshold cycle (CT) of each PCR product was defined as the cycle number at which the fluorescence signal passed the fixed threshold. Duplicate samples for each case were examined. The $2^{-\Delta t}$ method is a convenient technique to analyze relative changes in gene expression from real-time PCR experiments, as previously described (Livak and Schmittgen, 2001). The average CT value was calculated using G3PDH and ΔCT (average CT - average CT_{G3PDH}). The relative quantification of mRNA was calculated as $2^{-\Delta t}$ (Livak and Schmittgen, 2001).

Detection of splice variants of RHAMM

We subjected 50 ng of total RNA to RT reactions using a first-strand cDNA synthesis kit (Amersham Biosciences). To detect the 2 splice variants, $RHAMM^{-48}$ (48 bp deletion) and $RHAMM^{-147}$ (147 bp deletion), were used along with the oligonucleotide RT-PCR primers (Hokkaido System Science) listed in Table 1. First, we examined the expressions of the 2 splice variants and could only detect a single band in all cases, whereas most of the malignant tumor cell lines, such as

osteosarcoma cell lines U2OS and G292, showed 2 bands. The target sequence was amplified in a 50 μl reaction volume containing 1 μg cDNA, 0.2 mM dNTPs, 1.5 mM MgCl2, 0.2 μM of each primer and 1.0 U Platinum Taq (Invitrogen). PCR amplification consisting of 35 cycles (95°C for 1 minute, 57°C for 30 seconds, 72°C for 2 minutes) were performed after the initial Taq Gold activation step (94°C for 7 minutes). After PCR was completed, 10 μl of PCR product was analyzed by electrophoresis on 2% agarose gels.

Histological analysis

Tissue fragments obtained from the tumor cases were fixed in a 3.7% formaldehyde neutral buffer solution and embedded in paraffin, then 5 μm sections were prepared on silicon-coated glass slides and stained with hematoxylin-eosin for histological examinations. Some sections were stained for immunohistochemistry with an anti-RHAMM goat polyclonal antibody (Santa Cruz Biotechnology) and anti-Ki67 mouse monoclonal antibody (Immunotech). Endogenous peroxidase was quenched by incubating with 0.3% H_2O_2 in methanol for 30 minutes. The antigen retrieval pretreatments were subjected to pressure cooked for 10 minutes in 10 mM citrate buffer (pH 6.0). Non-specific staining was blocked using Dako Protein Block Serum Free (Dako). The sections were incubated with the primary antibody (1:100) overnight at 4°C, then incubated with the secondary antibody for 60 minutes. For visualization, the sections were treated with a Liquid DAB (3, 3'-diaminobenzidine) Chromogen System (Dako) according to the manufacturer's protocol, followed by counterstaining with haematoxylin.

Other sections were stained for immunofluorescence microscopy with an anti-RHAMM mouse monoclonal antibody (MONOSAN). Endogenous peroxidase was quenched by incubating with 0.3% H_2O_2 in methanol for 30 minutes. Non-specific staining was blocked using Dako Protein Block Serum Free (Dako). The sections were incubated with the primary antibody (1:100) overnight at 4°C. RHAMM staining was revealed by incubation with an Alexa-Fluor dye-labeled goat anti-mouse antibody (Invitrogen) for 60 minutes at room temperature. After 3 rinses in PBS, the slides were mounted in Vectashield (Vecto Laboratories) and examined using a Leica TCS STED (Leica Microsystems).

Statistical methods

Statistical analysis was performed using one-way ANOVA and Student's t-test. P values less than 0.05 were regarded as statistically significant.

Results

Clinical features

There were 65 patients with fibro-osseous lesions treated during the past 25 years at our department. Diagnoses were based on clinical, radiological and histological features, which identified ossifying fibroma in 12 patients (18%), fibrous dysplasia or cement-osseous dysplasia in 40 (62%), and oseteoblastoma, cementoblastoma or osteoma in 13 (20%) (Table 2). The average age of the patients was 35 years old (range, 18 to 62 years) and the male-female ratio was 1:3 (Table 2). These data concur with widely accepted data, showing

Table 1. Oligonucleotide primers sequences utilized for RT-PCR.

RT-PCR primer set			Sequence
RHAMM	-48bp	F	5'-ggccgtcaacatgtcctttccta-3'
		R	5'-ttgggctatttttcccttgagactc-3'
	exon 4	F	5'-caggtcacccaaaggagtctcg-3'
		R	5'-caagctcatccagtgtttgc-3'
	-147bp	F	5'-aggaggaacaagctgaaagg-3'
		R	5'-ttcctgagctgcaccatgtt-3'
ALP		F	5'-acgtggctaagaatgtcatc-3'
		R	5'-ctggtaggcgatgtcctta-3'
BSP		F	5'-ctatggagacgacgccacgc-3'
		R	5'-catagccatcgtagccttgtc-3'
OCN		F	5'-catgagagccctcaca-3'
		R	5'-agagcgacaccctagac-3'
G3PDH		F	5'-accacagtccatgccatcac-3'
		R	5'-tccaccaccctgtggctgta-3'

RHAMM, receptor for hyaluronan-mediated motility; ALP, alkaline phosphatase; BSP, bone sialoprotein; OCN, osteocalcin; G3PDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Clinical data for patients with fibro-osseous lesions.

	fibrogenic neoplasms	non-neoplastic bone lesions		osteogenic neoplasms		
	ossifying fibroma	fibrous dysplasia	cemento-osseous dysplasia	osteoblastoma	cementoblastoma	osteoma
age (mean)	18-62 (35)	14-87 (48)		18-79 (44)		
male:female	1:3	1:3.4		1:1.6		
number of cases	12	40		13		

y, year

that ossifying fibromas develop more often in females than males, and have a peak incidence during the third and fourth decades of life.

Expression of RHAMM mRNA in fibro-osseous lesions

Quantification of gene expression in formalin-fixed and paraffin-embedded (FFPE) tissues is well reported as compared to quantitative real-time RT-PCR findings. Results from a large number of studies can be used to establish practical recommendations for gene expression analysis of FFPE-derived RNA (Godfrey et al., 2000; Lewis et al., 2001), and suggest that mRNA expression levels in FFPE tissues reflect the actual expression level in the original tissue samples regardless of the method of fixation used (Farragher et al., 2008). Furthermore, G3PDH is a useful internal reference gene for quantitative real-time RT-PCR assays of gene expression in FFPE tissue samples (Mori et al., 2008).

We examined the expression of RHAMM mRNA in 65 fibro-osseous lesions and 5 samples from normal jaws using real-time RT-PCR. The mean expression level of RHAMM mRNA was much higher in the ossifying fibroma cases (11400±4500) as compared to that in the normal jaws (32.8±8.0) (Fig. 1).

Human RHAMM cDNA was cloned and found to contain 725 amino acids encoding an 84-kDa protein. Three distinct RHAMM gene products, full-length RHAMM, a splice variant with a 48-bp deletion ($RHAMM^{-48}$) and a variant with a 147-bp deletion ($RHAMM^{-147}$), were previously cloned from multiple myeloma cells (Wang et al., 1996). The expressions of these variants were examined in 65 fibro-osseous lesions using PCR amplification, which resulted in 2 products. Although malignant tumor cell lines from the sample osteosarcoma cell lines U2OS and G292 expressed $RHAMM^{-48}$ and $RHAMM^{-147}$, no expression could be identified in the 65 examined cases (data not shown). This suggests that expression of RHAMM variants may lead to malignancy and is the primary characteristic of an ossifying fibroma as a neoplasm.

Immunohistochemistry

Next, we examined the expression of RHAMM protein in the 65 cases using an immunohistochemical method. All of the ossifying fibroma specimens showed positive staining and RHAMM protein expression was detected in cells in the fibrous regions of the tissues (Fig. 2A,B), whereas those were not seen in most of the fibro-osseous lesions (Fig. 2C-F). Moreover, fibroblastic tumor cells revealed a markedly high expression of RHAMM, while osteoblastic cells surrounding calcified spherules such as ossicles and cementicles did not express RHAMM (data not shown). These results suggest that RHAMM expression more generally occurs in ossifying fibromas and plays an important role in the growth of fibroblastic cells and inhibition of osteogenesis. In addition, RHAMM expression may be linked to characteristic features of ossifying fibroma, including fibroblastic cellular-proliferation and differentiation.

Correlation between mRNA expression levels of RHAMM and osteogenenic markers

A total of 12 ossifying fibroma cases were investigated in regard to proliferation. First, we determined the approximate tumor size by multiplying the width, depth and height of lesions shown in X-ray images, which revealed 9 cases with markedly high levels of RHAMM mRNA that were also large in size (data not shown). However, it is debatable whether tumor size is dependent on the number of years after onset. We also investigated that Ki-67 index, which has been reported to be a good indicator of cell proliferation activity (Girod et al., 1993). RHAMM expression seemed to be correlated with the expression of Ki-67, though it was very low. Also, since ossifying fibromas are a type of neoplasm, Ki-67 expression was low. Our findings may support the notion that enhanced expression of RHAMM plays an important role in abnormal proliferation, though further examination is

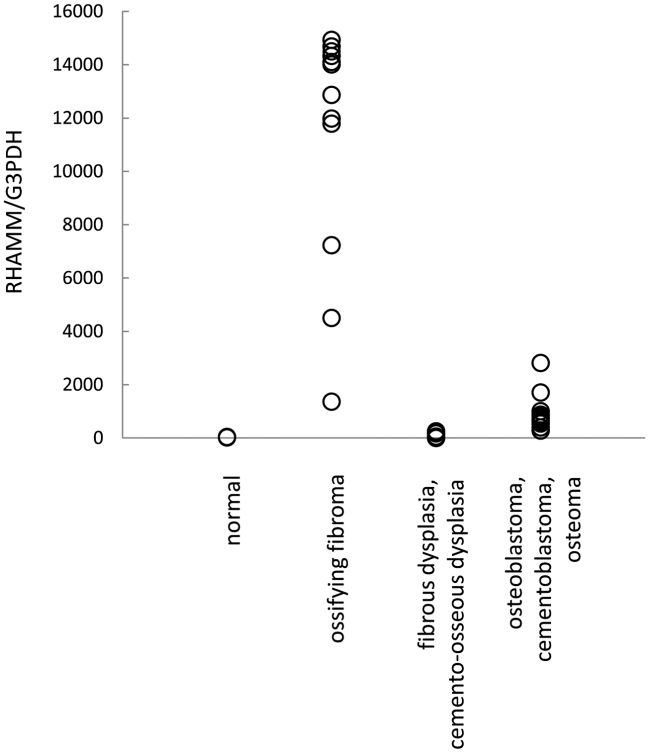

Fig. 1. Levels of RHAMM mRNA expression in fibro-osseous lesions and normal cases shown by quantitative RT-PCR analysis. Each point represents the expression of RHAMM mRNA. RHAMM mRNA expression levels were significantly higher in ossifying fibroma lesions than normal cases.

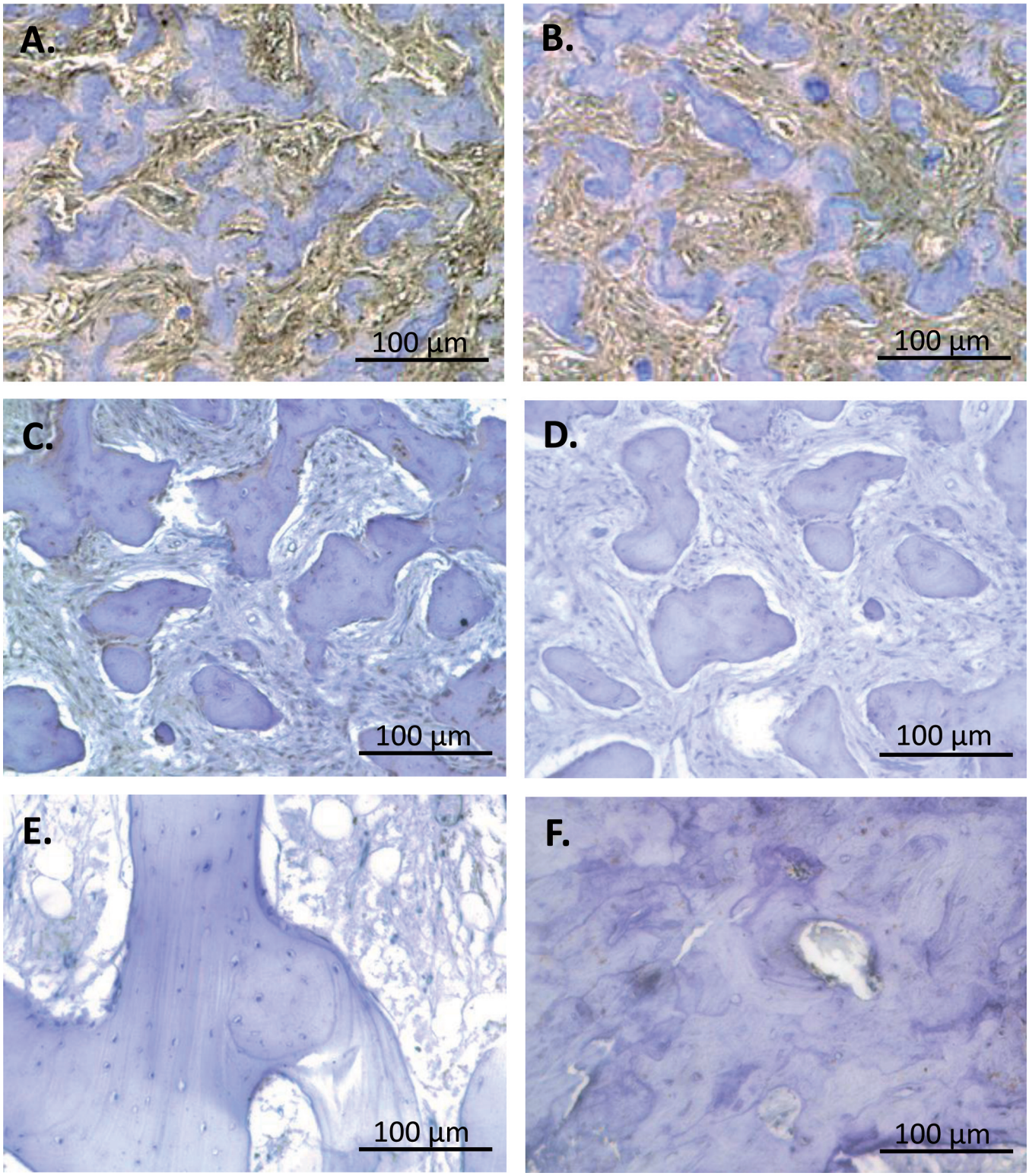

Fig. 2. Immunohistochemical findings for RHAMM in fibro-osseous lesion cases. All ossifying fibroma cases showed positive staining and the expression of RHAMM protein was detected in cells in the fibrous regions of the tumors. (**A and B**) ossifying fibroma cases. (**C and D**) dysplasia cases. (**E and F**) osteoma cases.

needed. We also found that RHAMM was associated with ERK, which was shown to promote the growth of immortalized human cementifying fibroma cell lines in our previous study (Hatano et al., 2011, 2012). Together, these findings indicate that RHAMM may be a fibroblastic cellular-proliferation factor of ossifying fibromas.

For normal jaw development, a balance of proliferation and differentiation of osteoblasts is important. We assumed that RHAMM overexpression would tip the balance toward proliferation in ossifying fibromas and may be linked to pathogenesis. We also examined mRNA expressions of osteogenic markers in 12 ossifying fibroma specimens using real-time RT-PCR. An inverse correlation was found between the mRNA expression level of RHAMM and osteogenic markers (Fig. 3). Our previous observations suggest a mechanism by which RHAMM/ERK functions as a negative regulator of mineralization in ossifying fibromas (Hatano et al., in press). In addition to its role in cell proliferation, RHAMM may serve as an anti-osteogenic factor in the osteolytic stage in ossifying fibroma tumors.

Discussion

Fibro-osseous lesions of the jaw are poorly understood. Treatment is usually a surgical procedure, thus identification of new diagnostic and prognostic markers is important to determine proper therapy.

Recently, responsible genes have been found in other studies of fibro-osseous lesions, which reported that ossifying fibromas may be correlated with *HRPT2* (Pimenta et al., 2006) and hyperparathyroidism-jaw tumor syndrome (HPT-JT) (Carpten et al., 2002). HPT-JT is an autosomal dominantly inherited disorder whose principal feature is the appearance of neoplastic and/or cystic lesions in 3 main organs; the parathyroid glands, jaws and kidneys (Jackson, 1958). Germline mutations of HRPT2 were previously found in 2 of 11 cases of familial isolated primary hyperparathyroidism and 1 of 2 families affected by HPT-JT (Mizusawa et al., 2006). Forty percent of individuals with HPT-JT may also develop jaw tumors, mainly ossifying fibromas (Chen et al., 2003). Although *HRPT2* is now widely accepted as the responsible gene for HPT-JT, some studies have pointed out that an *HRPT2* mutation is not common during development of an ossifying fibroma (Toyosawa et al., 2007). None of the ossifying fibroma patients in the present study developed HPT-JT symptoms or expressed *HRPT2* mutations (data not shown). Thus, most ossifying fibromas develop independently, while they sometimes occur in correlation with HPT-JT.

Among these different lesions, cases of ossifying fibroma are very difficult to accumulate because of their rarity, though it is important to study gene expression using FFPE tissues. On the other hand, isolation of high-quality genomic DNA from FFPE tissue samples is also difficult, since only minimal quantities of intact DNA are present in the samples. In this respect, identification of new markers that can be easily detected is important. With recent advancements, RNA from FFPE tissues of sufficient quality for gene expression can now be

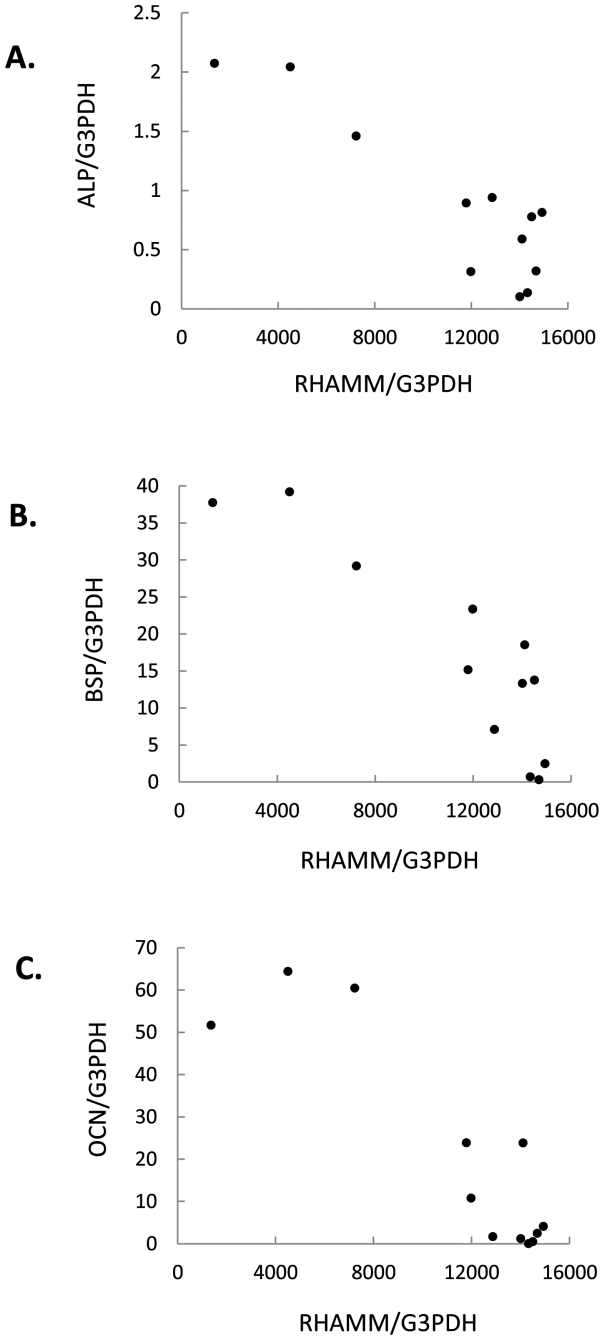

Fig. 3. Correlation between mRNA expression levels of RHAMM and osteogenic markers by RT-PCR. A correlation was found between the mRNA expression levels of RHAMM and osteogenic markers, as tumors that expressed high levels of RHAMM mRNA were low in mRNA expression of ALP, BSP, and OCN. **A.** ALP, alkaline phosphatase. **B.** BSP, bone sialoprotein. **C.** OCN, osteocalcin.

extracted, and have been used for both qRT-PCR and microarray analyses (Godfrey et al., 2000; Lewis et al., 2001; Farragher et al., 2008; Mori et al., 2008). There is fairly general consensus that qRT-PCR findings have approximately 90% concordance with microarray findings (Cronin et al., 2004).

Recently, we found RHAMM overexpression in established immortalized cell lines obtained from cases of human ossifying fibroma of the jaw (Hatano et al., 2011, 2012), indicating that RHAMM may be a candidate diagnostic marker. RHAMM mRNA expression has also been identified in normal human tissues, including those from the colon, stomach, ovary and testis (Turley et al., 1985), while its overexpression has been reported in several human cancers (Turley, 1982; Crainie et al., 1999; Wang et al., 1998; Rein et al., 2003; Lugli et al., 2006; Yamano et al., 2008). Therefore, up-regulation of RHAMM may be a common event in various neoplasms. In the present study, we investigated the expression of RHAMM mRNA in 65 fibro-osseous lesion cases, and found that it as well as protein expression were significantly higher in ossifying fibroma cases. We also examined the correlation between the expression of RHAMM mRNA and various disease characteristics.

In conclusion, the present results revealed that overexpression of RHAMM is related to the progression of ossifying fibroma. These findings provide new and important information regarding ossifying fibroma development, and suggest that RHAMM could be used as a novel molecular target for therapy in affected patients.

Acknowledgements. This work was supported by a Grant-in-Aid for JSPS fellows (No. 22-6035) from the Japan Society for the Promotion of Science (JSPS).

Conflicts of interest. The authors have no conflicts of interest to declare.

References

Barnes L., Eveson J.W., Reichart P., Sidransky D. and editors. (2005). WHO classification of tumors. Pathology and genetics of head and neck tumors. ARC Press. Lyon.

Brannon R.B. and Fowler C.B. (2001). Benign fibro-osseous lesions: a review of current concepts. Adv. Anat. Pathol. 8, 126-143.

Carpten J.D., Robbins C.M., Villablanca A., Forsberg L., Presciuttini S., Bailey-Wilson J., Simonds W.F., Gillanders E.M., Kennedy A.M., Chen J.D., Agarwal S.K., Sood R., Jones M.P., Moses T.Y., Haven C., Petillo D., Leotlela P.D., Harding B., Cameron D., Pannett A.A., Höög A., Heath H. 3rd, James-Newton L.A., Robinson B., Zarbo R.J., Cavaco B.M., Wassif W., Perrier N.D., Rosen I.B., Kristoffersson U., Turnpenny P.D., Farnebo L.O., Besser G.M., Jackson C.E., Morreau H., Trent J.M., Thakker R.V., Marx S.J., Teh B.T., Larsson C. and Hobbs M.R. (2002). HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. Nat. Genet. 32, 676-680.

Cerase A. and Priolo F. (1998). Skeletal benign bone-forming lesions. Eur. J. Radiol. 27, S91-97.

Chen J.D., Morrison C., Zhang C., Kahnoski K., Carpten J.D. and Teh B.T. (2003). Hyperparathyroidism-jaw tumour syndrome. J. Intern. Med. 253, 634-642.

Crainie M., Belch A.R., Mant M.J. and Pilarski L.M. (1999). Overexpression of the receptor for hyaluronan-mediated motility (RHAMM) characterizes the malignant clone in multiple myeloma: identification of three distinct RHAMM variants. Blood 93, 1684-1696.

Cronin M., Pho M., Dutta D., Stephans J.C., Shak S., Kiefer M.C., Esteban J.M. and Baker J.B. (2004). Measurement of gene expression in archival paraffin-embedded tissues: development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay. Am. J. Pathol. 164, 35-42.

Farragher S.M., Tanney A., Kennedy R.D. and Paul Harkin D. (2008). RNA expression analysis from formalin fixed paraffin embedded tissues. Histochem. Cell Biol. 130, 435-445.

Girod S.C., Krueger G. and Pape H.D. (1993). p53 and Ki 67 expression in preneoplastic and neoplastic lesions of the oral mucosa. Int. J. Oral Maxillofac. Surg. 22, 285-288.

Gitelis S. and Schajowicz F. (1989). Osteoid osteoma and osteoblastoma. Orthop. Clin. North. Am. 20, 313-325.

Godfrey T.E., Kim S.H., Chavira M., Ruff D.W., Warren R.S., Gray J.W. and Jensen R.H. (2000). Quantitative mRNA expression analysis from formalin-fixed, paraffin-embedded tissues using 5' nuclease quantitative reverse transcription-polymerase chain reaction. J. Mol. Diagn. 2, 84-91.

Hatano H., Shigeishi H., Kudo Y., Higashikawa K., Tobiume K., Takata T. and Kamata N. (2011). RHAMM/ERK interaction induces proliferative activities of cementifying fibroma cells through a mechanism based on the CD44-EGFR. Lab. Invest. 91, 379-391.

Hatano H., Shigeishi H., Kudo Y., Higashikawa K., Tobiume K., Takata T. and Kamata N. (2012). Overexpression of Receptor for Hyaluronan-Mediated Motility (RHAMM) in MC3T3-E1 induces the proliferation and differentiation through phosphorypation of ERK1/2. J. Bone Miner. Metab. 30, 293-303.

Jackson C.E. (1958). Hereditary hyperparathyroidism associated with recurrent pancreatitis. Ann. Intern. Med. 49, 829-836.

Kubista M., Andrade J.M., Bengtsson M., Forootan A., Jonák J., Lind K., Sindelka R., Sjöback R., Sjögreen B., Strömbom L., Ståhlberg A. and Zoric N. (2006). The real-time polymerase chain reaction. Mol. Aspects Med. 27, 95-125.

Kudo Y., Hiraoka M., Kitagawa S., Miyauchi M., Kakuo S., Zhao M., Ide T. and Takata T. (2002). Establishment of human cementifying fibroma cell lines by transfection with temperature-sensitive simian virus-40 T-antigen gene and hTERT gene. Bone 30, 712-717.

Lewis F., Maughan N.J., Smith V., Hillan K. and Quirke P. (2001). Unlocking the archive--gene expression in paraffin-embedded tissue. J. Pathol. 195, 66-71.

Livak K.J. and Schmittgen T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402-408.

Lugli A., Zlobec I., Günthert U., Minoo P., Baker K., Tornillo L., Terracciano L. and Jass J.R. (2006). Overexpression of the receptor for hyaluronic acid mediated motility is an independent adverse prognostic factor in colorectal cancer. Mod. Pathol. 19, 1302-1309.

Mizusawa N., Uchino S., Iwata T., Tsuyuguchi M., Suzuki Y., Mizukoshi T., Yamashita Y., Sakurai A., Suzuki S., Beniko M., Tahara H., Fujisawa M., Kamata N., Fujisawa K., Yashiro T., Nagao D., Golam H.M., Sano T., Noguchi S. and Yoshimoto K. (2006). Genetic

analyses in patients with familial isolated hyperparathyroidism and hyperparathyroidism-jaw tumour syndrome. Clin. Endocrinol. (Oxf) 65, 9-16.

Mori R., Wang Q., Danenberg K.D., Pinski J.K. and Danenberg P.V. (2008). Both beta-actin and GAPDH are useful reference genes for normalization of quantitative RT-PCR in human FFPE tissue samples of prostate cancer. Prostate 68, 1555-1560.

Paul M.S. and Roman C. (2006). Maxillofacial fibro-osseous lesions. Curr. Diagn. Pathol. 12, 1-10.

Pimenta F.J., Gontijo Silveira L.F., Tavares G.C., Silva A.C., Perdigão P.F., Castro W.H., Gomez M.V., Teh B.T., De Marco L. and Gomez R.S. (2006). HRPT2 gene alterations in ossifying fibroma of the jaws. Oral Oncol. 42, 735-739.

Rein D.T., Roehrig K., Schöndorf T., Lazar A., Fleisch M., Niederacher D., Bender H.G. and Dall P. (2003). Expression of the hyaluronan receptor RHAMM in endometrial carcinomas suggests a role in tumour progression and metastasis. J. Cancer Res. Clin. Oncol. 129, 161-164.

Slootweg P.J. (1996). Maxillofacial fibro-osseous lesions: classification and differential diagnosis. Semin. Diagn. Pathol. 13, 104-112.

Su L., Weathers D.R. and Waldron C.A. (1997). Distinguishing features of focal cemento-osseous dysplasia and cemento-ossifying fibromas. II. A clinical and radiologic spectrum of 316 cases. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 84, 540-549.

Toyosawa S., Yuki M., Kishino M., Ogawa Y., Ueda T., Murakami S., Konishi E., Iida S., Kogo M., Komori T. and Tomita Y. (2007). Ossifying fibroma vs fibrous dysplasia of the jaw: molecular and immunological characterization. Mod. Pathol. 20, 389-396.

Turley E.A. (1982). Purification of a hyaluronate-binding protein fraction that modifies cell social behavior. Biochem. Biophys. Res. Commun. 108, 1016-1024.

Turley E.A., Bowman P. and Kytryk M.A. (1985). Effects of hyaluronate and hyaluronate binding proteins on cell motile and contact behaviour. J. Cell Sci. 78, 133-145.

Waldron C.A. (1993). Fibro-osseous lesions of the jaws. J. Oral Maxillofac. Surg. 51, 828-835.

Wang C., Entwistle J., Hou G., Li Q. and Turley E.A. (1996). The characterization of a human RHAMM cDNA: conservation of the hyaluronan-binding domains. Gene 174, 299-306.

Wang C., Thor A.D., Moore D.H. 2nd, Zhao Y., Kerschmann R., Stern R., Watson P.H. and Turley E.A. (1998). The overexpression of RHAMM, a hyaluronan-binding protein that regulates ras signaling, correlates with overexpression of mitogen-activated protein kinase and is a significant parameter in breast cancer progression. Clin. Cancer Res. 4, 567-576.

Yamano Y., Uzawa K., Shinozuka K., Fushimi K., Ishigami T., Nomura H., Ogawara K., Shiiba M., Yokoe H. and Tanzawa H. (2008). Hyaluronan-mediated motility: a target in oral squamous cell carcinoma. Int. J. Oncol. 32, 1001-1009.

Accepted October 8, 2012