J Neurooncol (2014) 116:77–82
DOI 10.1007/s11060-013-1274-4

LABORATORY INVESTIGATION

Expression of insulin-like growth factor II mRNA-binding protein 3 (IMP3) in sacral chordoma

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Received: 11 April 2013 / Accepted: 9 October 2013 / Published online: 18 October 2013

Abstract Sacral chordoma is a rare and aggressive tumor, with a high rate of local recurrence even when the tumor is radically resected. The fundamental knowledge of its biological behavior remains unknown. Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is one of the RNA binding proteins and is expressed during embryogenesis and in various malignant tumors. This study evaluated expression of IMP3 in sacral chordoma for association with patient's clinicopathological factors. A total of 32 patients with sacral chordoma (17 male and 15 female) and 10 samples of distant normal tissues were collected for analysis of IMP3 expression using immunohistochemistry. Association between IMP3 expression and clinicopathological factors (such as patient's age, gender, tumor location, tumor size, surrounding muscle invasion, Ki-67 expression, and tumor recurrence) were statistically analyzed. IMP3 was expressed in 20 (62.5 %) patients, whereas there was no expression in the 10 distant normal tissues. IMP3 expression was associated with tumor invasion into the surrounding muscle ($P = 0.028$), high levels of Ki-67 expression ($P = 0.009$), and tumor recurrence ($P = 0.012$). The log-rank test revealed that patients with positive IMP3 expression had a shorter continuous disease-free survival time than those with negative IMP3 expression ($P = 0.016$). IMP3 expression was independent of age, gender, tumor location and tumor size. These results indicate that IMP3 was overexpressed in sacral chordoma and this expression was associated with tumor invasion and recurrence; thus, IMP3 may play an important role in tumor progression and could serve as a prognostic biomarker for sacral chordoma and IMP3 could be used as a potential therapeutic target for the treatment of sacral chordoma.

Keywords Chordoma · Sacral · Immunohistochemistry · IMP3 · Tumor recurrence · Survival

Introduction

Chordoma is relatively rare, slow growing, and locally aggressive low to intermediate-grade malignant bone tumor, which is thought to originate from the notochordal remnants. The incidence rate is approximately 0.08 per 100,000 annually [1]. Chordoma commonly occurs at the sacrum, accounting for approximately 50–60 % of chordoma along the axial skeleton, and is characterized by invasive and destructive growth patterns [2]. Due to their indolent and low-grade nature, sacral chordoma will often have achieved a large size by the time of diagnosis. These tumors are minimally responsive to radiation and chemotherapy and surgical resection remains the curable treatment [3] but a high recurrence rate does occur even after the tumor has been radically resected, leading to a poor outcome and quality of life [4, 5]. Taken altogether, this aggressive biological behavior contributes to the high recurrence rate of this tumor type [6]. Thus, novel

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approaches are urgently required to accurately assess individual patient risk of tumor progression and response to treatment. Identification of novel biomarkers could help to predict recurrence and survival rates of the patients and may also provide a target for tumor therapy.

Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is a member of the insulin-like growth factor II (IGF2) mRNA binding protein (IMP) family [7], which plays an important role in RNA trafficking and stabilization, and cell growth and migration during the early stages of embryogenesis [8]. IMP3 is also known as L523S or KOC (K homologous domain-containing protein overexpressed in cancer), a protein containing four K homology domains and two other RNA recognition motifs [9, 10]. IMP3 is located on chromosome 7p11.2 and is identical to the KOC protein that was originally cloned from a pancreatic tumor cDNA screen [11]. Recent studies have demonstrated that IMP3 was expressed in various cancers, including cancers of the pancreas, kidney, bladder, and colon and in osteosarcoma, but was not detectable in the adjacent benign tissues [10, 12–15]. IMP3 expression has also been associated with metastasis of renal cell carcinoma and progression of colon cancer [12, 14]. Thus, in the present study, we investigated IMP3 expression in sacral chordoma and its association with clinicopathological data and survival of the patients.

Materials and methods

Patients and tissue samples

Thirty-two patients (17 males and 15 females) with sacral chordoma were retrospectively identified and these patients had been surgically treated at the Department of Orthopedic Surgery, The First Affiliated Hospital of Soochow University, between January 2000 and March 2011. The average age at the time of surgery was 51.2 ± 13.7 years. Clinicopathological and survival data were obtained from their medical records, such as age, gender, tumor location, tumor size, and tumor invasion into the surrounding muscle. The tumor invasion into the surrounding muscle was confirmed by preoperative magnetic resonance (MR) images and pathology examinations. This study was approved by our hospital review board and each patient signed an informed consent form.

Surgical tissue samples were fixed in formalin and embedded in paraffin. For this study, we retrospectively retrieved these paraffin blocks, which contained tumor tissues from all 32 patients and distant normal tissue samples from 10 patients, which were at least 3 cm away from the lesions. Histologic sections were prepared and stained with hematoxylin and eosin for further confirmation of histology diagnosis by two pathologists who confirmed the diagnosis of these patients.

Immunohistochemistry

Immunohistochemical staining was performed with the EnVision two-step staining method on 4-μm-thick tissue sections. Briefly, the sections were dewaxed in xylene and rehydrated in a series of ethanol before the antigen retrieval. The primary antibodies used were IMP3 (ab126082; Abcam, Cambridge, MA, USA) at a dilution of 1:100 and Ki-67 (GM724002; Gene Tech, Shanghai, China) at a dilution o f 1:100. The ChemMate^{TM} Envision^{TM} Detection Kit was used from Gene Tech (GK500710) according to the manufacturer's protocol. The antibody binding was visualized by using 3,3′-Diaminobenzidine solution and briefly counterstained with hematoxylin. For positive control, tissue sections of cervical carcinoma with known positivity were used in each batch of staining and for negative control, the primary antibody was replaced by non-immune IgG. Positive IMP3 staining was shown in the nuclei and cytoplasm by a brown color. These immunostained tissue sections were then reviewed and scored under a microscope independently by two pathologists who had no prior knowledge of the patients' clinicopathological data or outcome as described previously [14], i.e., five high power fields were randomly selected and evaluated semiquantitatively in combination with staining intensity and % of staining. Staining intensity for IMP3 was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). % of staining was scored as 0 (0 %), 1 (1–25 %), 2 (26–50 %), 3 (51–75 %), and 4 (76–100 %). The sum of the staining intensity and % of staining scores was used as the final staining score. The specimens were divided into two groups according to their overall scores: 0–1, negative; 2–7, positive. Ki-67 index was scored as the percentage of cells with positive nuclear staining, and samples were divided into two groups: low (<10 % of cells with positive nuclei) and high (>10 % of cells with positive nuclei).

Patients' follow-up

Patients were followed up regularly, which included plain radiographs, computed tomography scans, and MR imaging every 3 months during the first 2 years and then at 6-monthly intervals after 3 years. Continuous disease-free survival (CDFS) was defined as the period of time between the primary surgery and tumor recurrence.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL). A χ^2 test or

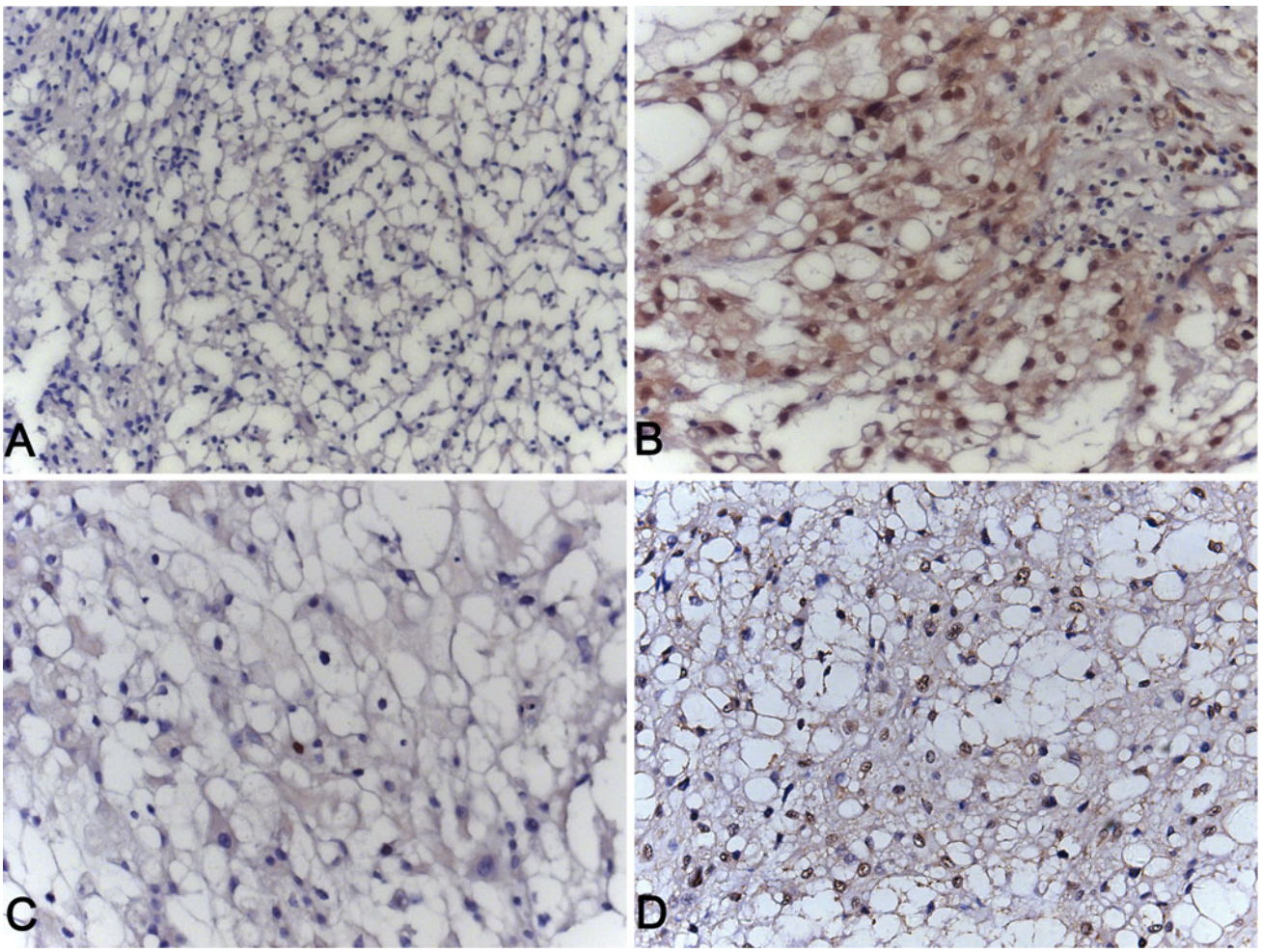

Fig. 1 Immunohistochemical analysis of IMP3 expression. **a** Negative expression of IMP3 in sacral chordoma. **b** Positive expression of IMP3 in sacral chordoma. **c** Low expression of Ki-67 in sacral chordoma. **d** High expression of Ki-67 in sacral chordoma. All magnification, ×400

Fisher's exact test was used, as appropriate, to analyze the association between IMP3 and clinicopathological variables. A Spearman rank correlation test was employed to analyze the correlation between expression of IMP3 and Ki-67. CDFS was estimated by using Kaplan–Meier survival curves, and a Log-rank test was used to evaluate the influences of IMP3 expression on CDFS. A P value less than 0.05 was considered to be statistically significant.

Results

In this study, we analyzed IMP3 expression in 32 surgical tissue specimens from 32 patients with sacral chordoma using immunohistochemical analysis and found that 20 of the 32 (62.5 %) tumor tissues expressed IMP3 protein, whereas none of the 10 distant normal tissues expressed IMP3 protein. IMP3 protein was stained positively in the nuclei and cytoplasm of the tumor cells with diverse intensity (Fig. 1). Statistical analysis revealed that expression of IMP3 protein was significantly higher in sacral chordoma than in distant normal tissues ($P = 0.001$; Table 1). IMP3 expression was associated with tumor invasion into the surrounding muscle ($P = 0.028$) and high expression of Ki-67 ($P = 0.009$), suggesting a potential role for IMP3 in promotion of tumor cell invasion. However, IMP3 expression was not statistically associated with age, gender, tumor size, nor tumor location (Table 2).

Table 1 Expression of IMP3 in sacral chordoma and distant normal tissue samples

Tissue sample	n	IMP3 expression		P-value
		Positive (%)	Negative (%)	
Sacral chordoma	32	20 (62.5)	12 (37.5)	0.001
Distant normal tissue	10	0 (0)	10 (100)	

Furthermore, we obtained follow-up data for all of the patients, i.e., the median follow-up time was 110 months

Table 2 Association of IMP3 expression with clinicopathological parameters in sacral chordoma

Parameters	*n*	IMP3 expression		*P*-value
		Positive	Negative	
Age (years)				0.291
<50	15	11	4	
≥50	17	9	8	
Gender				0.291
Male	17	9	8	
Female	15	11	4	
Tumor location				1.000
Above S3	17	11	6	
S3 and below	15	9	6	
Tumor size (mm)				0.713
<90	13	9	4	
≥90	19	11	8	
Surrounding muscle invasion				0.028
Yes	14	12	2	
No	18	8	10	
Ki-67				0.009
High	22	17	5	
Low	10	3	7	
Recurrence				0.012
Yes	15	13	2	
No	17	7	10	

(ranged between 24 and 157 months), and 15 (46.9 %) patients developed local recurrence, with a median recurrence time of 35 months (ranging from 3 to 92 months). The Kaplan–Meier survival curve analysis showed that CDFS rates were 71.3 and 64.5 % for 3 and 5 years, respectively. In patients with negative IMP3 expression, only 2 out of the 12 had recurrence, whereas 13 out of the 20 patients with IMP3 expression had recurrence. The log-rank test revealed that patients with IMP3 expression had a shorter CDFS than those with negative IMP3 expression (median 51 vs. 86.5 months, $P = 0.016$; Fig. 2).

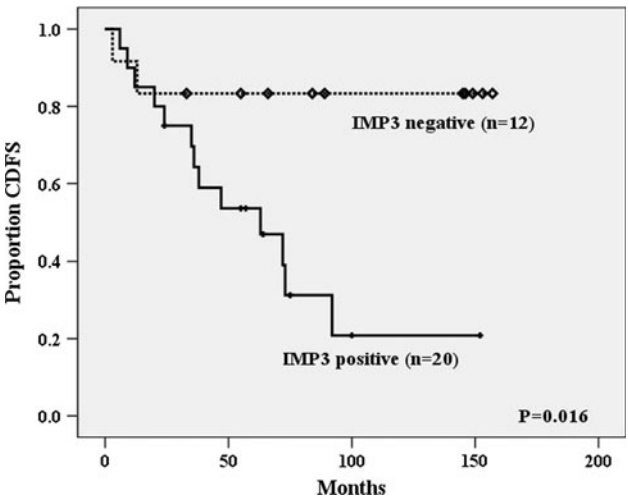

Fig. 2 Kaplan–Meier survival curve analysis of CDFS according to IMP3 expression

Discussion

In the current study, we detected IMP3 expression in sacral chordoma tissue specimens and investigated association with clinicopathological and survival data. Our data showed that IMP3 protein was overexpressed in sacral chordoma tissues compared to the distant normal tissues. Overexpression of IMP3 protein was associated with tumor cell invasion into the surrounding muscle, tumor reoccurrence, and high expression of Ki-67. Moreover, patients with IMP3 expression had a shorter CDFS than those with negative IMP3 expression. These data indicate that detection of IMP3 could be useful in prediction of tumor reoccurrence and survival of the patients.

Sacral chordoma is relatively rare and slow growing, but a locally aggressive bone tumor. Clinically, it shows frequent reoccurrence after radical resection of tumor lesions [16]. Many clinicopathological factors, such as tumor location and size, type of surgery, surgical margins, tumor invasion into the surrounding muscle, and expression of MMP-9, have been reported to be potential prognostic factors for patients after surgery [17–20]. Due to its rarity, definitive characteristics of this disease have not been defined and its biological behavior remains largely unknown [18]. In the current study, we showed that IMP3 was expressed in 62.5 % of sacral chordoma tissue samples, but was not in the distant normal tissues, which is consistent with previous studies in other types of human cancers [10, 12–15]. IMP3 has been shown to be useful as a novel biomarker to differentiate normal from cancerous tissues in a variety of organ systems [21]. Thus, IMP3 could be further evaluated as a diagnostic marker for sacral chordoma.

Furthermore, our current study showed a significant association of elevated IMP3 expression with tumor invasion into the surrounding muscle. IMP3 was previously reported to be associated with advanced clinical stage, increased regional lymph node involvement, and distant metastases of renal cell carcinoma [12]. IMP3 has also been shown to promote cell adhesion and invadopodia formation in HeLa cells and overexpression of IMPs in cancer cells may promote their invasive capacity [22]. It has also been reported that IMP3 has contributed in vitro to breast cancer cell migration and invasion and was an effector of EGFR-mediated migration and invasion [23]. Previous studies have also shown that expression of IMP3

is associated with tumor cell proliferation, adhesion, and invasion [22, 24]. An in vitro study showed that IMP3 was able to enhance cell proliferation [25]. Our current study demonstrated that IMP3 expression was associated with Ki67 overexpression in sacral chordoma tissues. This is in accordance with other studies that have found that IMP3 expression is associated with Ki-67 expression in colon cancer [14]. Ki-67 is a proliferation-associated antigen and expressed at all stages of mitosis except during the G0 phase of the cell cycle. Taken together this suggests that IMP3 plays a critical role in tumor progression and metastasis; thus, detection of IMP3 expression could be used to predict aggressiveness and reoccurrence of chordoma.

Sacral chordoma often recurs despite optimal therapy, and local recurrence is the most important predictor of mortality in patients with sacral chordoma [26]. In our current study, the log-rank test revealed that CDFS was significantly shorter in patients with IMP3 expression than in patients without IMP3 expression (median 51 vs. 86.5 months, $P = 0.016$). In other tumor types, IMP3 has been reported to be a prognostic factor [21]. Lochhead et al. [27] showed that IMP3 expression was associated with poorer survival in patients with colorectal cancer, independent of *BRAF* mutation and *LINE-1* hypomethylation. Chen et al. [28] reported that IMP3 expression predicted early tumor recurrence and was a strong indicator of worse disease-free survival. These results indicate that IMP3 is an oncoprotein and contributes to tumor progression.

Based on published data and our current data, IMP3 may be a potential therapeutic target [9]. In the study by Suda et al. [29], cytotoxic T cell clones were generated by in vitro exposure to a human leukocyte antigen (HLA)-A24-restricted peptide epitope derived from IMP3, and were able to induce potent and specific immune response against lung and esophageal cancer cells that express IMP3. In a phase I clinical trial, vaccination therapy using an IMP3-derived peptide, together with two other peptides, was administered to 10 esophageal cancer patients who were resistant to chemotherapy and/or radiotherapy and the data showed satisfactory immunogenicity and disease control rates [30]. Recently, these investigators performed a phase II clinical trial and demonstrated that vaccination-induced immune response positively correlated with a better prognosis for patients with advanced esophageal squamous cell carcinoma [31]. These data support the potential of anti-IMP3 therapy as a novel adjuvant treatment for human cancer, and subsequently, may also be useful in future treatment regimes for patients with sacral chordoma. However, our current study is just a proof-of-principle and further research is required to verify the role of IMP3 in the progression of sacral chordoma.

Acknowledgments We would like to thank Gu Yongping for technical guidance during the immunohistochemical analysis.

Conflict of interest There are no conflicts of interest to be declared for this work.

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