Virchows Arch (2015) 467:329–337
DOI 10.1007/s00428-015-1799-3

ORIGINAL ARTICLE

In malignant cartilagenous tumors, immunohistochemical expression of procollagen PC1CP peptide is higher and that of PC2CP lower than in benign cartilaginous lesions

Camille Delaunay-Lemarie[1] · Jean-Baptiste Vincourt[2] · Béatrice Marie[1] · Shyue-Fang Battaglia-Hsu[3,4] · Stéphanie Etienne[2] · François Sirveaux[5] · Phi Linh Nguyen Thi[6] · Jacques Magdalou[2] · Jean-Michel Vignaud[1,4,7] · Guillaume Gauchotte[1,4,8]

Received: 20 February 2015 / Revised: 12 May 2015 / Accepted: 8 June 2015 / Published online: 23 June 2015

Abstract Few studies on oncogenesis of chondrosarcoma (CS) are available in the literature. Our previously published experimental evidence suggests that while the C-propeptide of procollagen Iα1 (PC1CP), a component of cartilage, favors tumor progression, the C-propeptide of procollagen IIα1 (PC2CP) exerts antitumor properties. In this study, we analyzed expression of PC1CP and PC2CP by immunohistochemistry in a series of enchondromas and CS. Our retrospective series consisted of 88 cases, including 43 CSs, 34 enchondromas and 11 nontumor samples. Immunohistochemical staining for PC1CP and PC2CP was evaluated in the cytoplasm and in the extracellular matrix (ECM). Diffuse staining for PC1CP in ECM was significantly more frequent in tumor than in nontumor samples (32 % vs. 0 %; $p = 0.03$), and in CSs than in enchondromas (44 vs. 18 %; $p = 0.02$). ECM semiquantitative score was higher in tumors than in nontumor samples ($p < 0.005$) and higher in CSs than in enchondromas ($p = 0.05$). Staining for PC2CP in ECM was more frequently found in enchondromas than in CSs (59 vs. 33 %; $p = 0.02$). ECM semiquantitative score was higher in enchondromas than in CSs ($p = 0.02$). Diffuse staining for PC1CP in combination with absence of staining for PC2CP had 94 % specificity for CS but with a sensitivity of only 35 %. Expression of neither PC1CP nor PC2CP correlated with recurrence-free survival or occurrence of metastases. In conclusion, we show that the expression of PC1CP is higher and that of PC2CP lower in malignant cartilaginous tumors. These results support an oncogenic role of PC1CP and anti-oncogenic property of PC2CP in cartilaginous tumors.

Keywords PC1CP · PC2CP · C-propeptides of procollagens · Enchondroma · Chondrosarcoma

Jean-Michel Vignaud and Guillaume Gauchotte contributed equally to this work.

✉ Guillaume Gauchotte
g.gauchotte@chu-nancy.fr

[1] Department of Pathology, CHU, Nancy, France

[2] UMR 7561 CNRS, Faculty of Medicine, Université de Lorraine, Vandoeuvre-lès-Nancy, France

[3] Department of Biochemistry and Molecular Biology, CHU, Nancy, France

[4] INSERM U954, Faculty of Medicine, Université de Lorraine, Vandoeuvre-lès-Nancy, France

[5] Department of Orthopedics, Centre Chirurgical Emile Gallé, Nancy, France

[6] ESPRI - Clinical Epidemiology and Evaluation Department, CHU, Vandoeuvre-lès-Nancy, France

[7] Centre de Ressources Biologiques BB-0033-00035, CHU, Nancy, France

[8] Service d'Anatomie et Cytologie Pathologiques, Hôpital Central, CHU de Nancy, 29, avenue du Maréchal de Lattre de Tassigny, 54000 Nancy, France

Introduction

Chondrosarcoma (CS) is a malignant cartilage matrix-producing neoplasm. Primary (conventional) CS is the third most common primary bone malignancy and represents more than 90 % of CSs [1]. WHO 2013 grading (ranging to I to III) is an important prognostic factor in primary CS [1]. Most cases of grade I CS, the distinction of which from enchondroma is often difficult [2], have a benign course. On

the contrary, grade II and III CS are more aggressive and therefore require more intensive treatment [1].

Karyotypes of most CS are complex but nonspecific and its oncogenesis is poorly understood [3–7]. Few studies have addressed the potential role played by extracellular matrix (ECM), such as aberrant distribution of heparan sulfate proteoglycans and deregulation of collagens I, II, III, and X, in the development of CSs [3, 8–13]. To better understand its oncogenesis, we previously compared proteomes of enchondromas and CSs of all types and grades, in an attempt to isolate proteins specifically associated with oncogenesis of chondrogenic tumors [14]. Proteomics and Western blot analyses showed that the COOH-terminal propeptides of procollagen Iα1 (PC1CP) and IIα1 (PC2CP) are differentially expressed in malignant vs. benign tumors, with increased PC1CP and decreased PC2CP expression significantly associated with CS. We subsequently studied in vitro the role recombinant soluble or immobilized PC1CP and PC2CP plays in the extracellular matrix. As these two peptides induced β1 integrin-mediated chondrocyte adhesion by distinct domains with variable efficacy, we suggested that distinct signaling pathways are involved. Immobilized PC2CP but not PC1CP induced apoptosis in chondrocytes and EAhy926 endothelial cells, while soluble PC1CP but not PC2CP induced migration of EAhy926 cells and upregulated expression of both vascular endothelial growth factor (VEGF) and CXCR4 in chondrocytes. Although soluble PC2CP also increased VEGF expression, expression of CXCR4 and of matrix metalloproteinase 13 was more pronounced. This experimental evidence suggested that PC1CP favors angiogenesis and tumor progression, but immobilized PC2CP blocks angiogenesis and reduces tumor progression via apoptosis while soluble PC2CP favors tumor progression and metastasis [14]. These results suggested that detection of these peptides might have potential diagnostic and prognostic use.

The aim of this study was to characterize expression of PC1CP and PC2CP by immunohistochemistry in a large series of enchondromas and CSs as well as in normal and arthritic cartilage, in order to elucidate their potential role as diagnostic and prognostic marker.

Materials and methods

Population and clinical data

Our study cohort consisted of 88 cases, retrospectively retrieved from the files of the Department of Pathology of Nancy (CHU) between 2000 and 2009, including 43 chondrosarcomas (grade I, 10/43; grade II, 24/43; grade III, 5/43; dedifferentiated, 4/43), 34 enchondromas, 5 samples of arthritic cartilage, and 6 samples of normal cartilage.

Anonymity of the patients was strictly respected, following local ethical guidelines (institutional review board DC2008-459). All specimens were reviewed by two experienced bone pathologists (JMV, BM) in order to confirm the initial diagnosis, according to the criteria of the 2013 WHO classification [1]. The correlation between pathological diagnosis and clinical and radiological features was systematically reviewed during multidisciplinary staff meetings. A representative paraffin block was selected from each case.

Clinical data, including age, sex, localization, treatment and survival, were collected from patient files in the Department of Orthopedics.

Immunohistochemistry

Paraffin sections (5 μm) were dewaxed in Tissue Clear (Medite) during 10 min and rehydrated (ethanol 100, 95, 70°, distilled water). Proteoglycans were then digested with hyaluronidase (500 U/mL) and chondroitinase ABC (1 U/mL) (Sigma) during 2 h at 37 °C. Antigen retrieval was performed by heating (30 min at 65 °C) in 10 mM sodium citrate buffer (pH 6).

Chicken anti-PC1CP and rabbit anti-PC2CP were generated at Eurogentech (Liège, Belglium) using the following peptides as immunogens: NH2-CWYISKNPKDKRHXWF-COOH (PC1CP), and NH2-SSKSKEKKHIWFC-COOH and NH2-ADQAAGGLRQHDAECCOOH (PC2CP). Specificity of the antibodies was verified by Western blotting, which showed a single band at expected position of 34 KDa for both PC1CP and PC2CP, and by immunohistochemistry using 28 nontumor and tumor samples [14].The following dilutions were used: PC1CP, 1/200; PC2CP, 1/200. Immunohistochemistry was performed with a Dako Autostainer Plus (Dako, Glostrup, Denmark) with LSAB + system-HRP K0679 revelation system (Dako), using biotin-streptavidin amplification and diaminobenzidine as a chromogen, following the manufacturer's instructions. As positive control, we used chondroma and CS samples for which expression of PC1CP and PC2CP had been confirmed by Western blot. As negative controls, we used tissue sections digested with hyaluronidase and chondroitinase and omitting either the primary or the secondary antibody [14]. Differential expression of PC1CP and PC2CP had been previously assessed by two-dimensional electrophoresis and mass spectrometry fingerprinting [14].

Staining of extra-cellular matrix (ECM) was evaluated using a semiquantitative score of 0–12, calculated by multiplying a distribution score by an intensity score. The distribution score was based on the percentage of ECM surface area stained: 1 for 0–5 % of surface area stained, 2 for 6–25 %, 3 for 26–50 %, and 4 for a surface area larger than 50 %. The intensity score ranged from 0 to 3, 0 for no staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining. Cytoplasmic staining was scored as positive (when at least 1 % of the cells stained) or negative (less than 1 %), and focal

(when less than 50 % of the cells stained) or diffuse (more than 50 %). Because in the dedifferentiated component of the dedifferentiated CSs no staining for PC1CP or PC2CP was found, staining was only evaluated in the chondroid component of these tumors. Scoring was performed independently by two observers (CDL, JMV). In case of discrepancy, a consensus score was established after discussion.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 23.0 (International Business Machines Corp., New York, USA). For qualitative variables, distribution among the groups was compared using chi-square test, except when any included data point had an expected frequency of less than 5, in which case Fisher's exact test was used. For qualitative variables, the Kruskal-Wallis test was used to compare the distribution among the three groups, and Mann Whitney *U* test to compare between nontumor vs. tumor and chondroma vs. CS groups. Survival analysis was performed in the CS group, with log-rank test for qualitative variables (ECM diffuse staining vs. focal staining for PC1CP; ECM negative staining vs. positive staining for PC2CP), and Cox model for PC1CP and PC2CP ECM score. A p value of less than 0.05 was considered to be statistically significant.

The level of agreement between the two assessors was measured with the weighted Kappa coefficient for distribution and intensity of PC1CP and PC2CP ECM scores; the non-weighted Kappa coefficient was used for the qualitative binary evaluation of cytoplasmic immunoreactivity, and the intraclass correlation coefficient (ICC) was used for PC1CP and PC2CP semiquantitative ECM scores using the MedCalc Statistical Software version 15.2.2 (MedCalc Software bvba, Ostend, Belgium) (95 % confidence interval [95 % CI]).

Sensitivity and specificity for a diagnosis of malignancy were calculated for each marker separately and in combination, considering grade I CS alone, or both grade I and II CSs.

Results

Clinical data

Clinical data are summarized in Table 1. Patients with CS (n = 43) had a mean age of 57 years, significantly higher than those with chondroma (mean, 37 years; $p < 0.0001$). In the chondroma group, four patients had Ollier disease and one patient Maffucci syndrome. In the CS group, four patients had Ollier disease. Most chondromas occurred in long bones (62 %), followed by extremities (hands or feet) (35 %), whereas the most frequent localization of CS was in long bones (44 %), followed by pelvis (16 %), ribs (12 %), and craniofacial bones (12 %; ethmoid,two cases and mandibular,three cases). All patients were treated with surgery (intralesional curettage for chondromas), associated with radiotherapy in 12 CS cases and chemotherapy in 3 CS cases. Only one patient with chondroma had a relapse. In the CS group, local relapses occurred in 12 cases, metastases in 13 cases, and seven patients died of disease (5 year overall survival: 82 %).

PC1CP

ECM from the superficial layer of normal cartilage stained for PC1CP (Fig. 1) along with either weak or absent cytoplasmic staining in chondrocytes. The same staining pattern occurred in the cartilage of arthritic tissues but extending further to the intermediate layers. In contrast, in 38 % of enchondromas and 56 % of CSs tumor chondrocytes expressed a significantly higher level of PC1CP, with diffuse cytoplasmic staining in 12 % of enchondromas and 33 % of CS (p = 0.02) (Table 2).

Diffuse PC1CP staining (score 4, i.e., greater than 50 % surface) of ECM was significantly more frequent in tumors than in non tumor samples (32 vs. 0 %; p = 0.03), and in CSs than in enchondromas (44 vs. 18 %; p = 0.02). It was also more frequent in grade II or III or dedifferentiated CSs than in enchondromas and grade I CSs (42 vs. 20 %; p = 0.04). No significant difference was detected between enchondromas and grade I CSs. ECM semiquantitative score was higher in tumors than in nontumor samples ($p < 0.005$), and higher in CSs than in enchondromas (p = 0.05) (Fig. 2). No correlation with recurrence-free survival or metastases was found.

The inter-assessor agreement was good for the evaluation of PC1CP ECM distribution (Kappa coefficient, 0.63; 95 % CI, 0.51–0.76), intensity (Kappa, 0.64; 95 % CI, 0.51–0.77), and semiquantitative scores (ICC, 0.79; 95 % CI, 0.69–0.86) and was moderate for the detection of PC1CP cytoplasmic immunoreactivity (0.47; 95 % CI, 0.31–0.63).

PC2CP

In normal-appearing cartilage, staining for PC2CP was detectable in five out of the six cases studied, with weak ECM (Fig. 3) and diffuse cytoplasmic staining in 50 % of cases. The five arthritic samples showed heterogeneous PC2CP staining of ECM.

Tumor cells cytoplasm was positive for PC2CP in 97 % of enchondromas and 91 % of chondrosarcomas, without a significant difference between these two groups (Table 2).

PC2CP distribution in ECM was significantly different between non tumor cartilage, chondromas, and CSs groups (p = 0.001; Kruskal-Wallis test). Most notably, PC2CP staining for ECM (distribution score 2, 3, or 4; i.e., greater than 5 %) was more frequently found in enchondromas than in chondrosarcomas (59 vs. 33 %; p = 0.01) (Fig. 3), and in grade I CS and enchondromas than in grade II and III CS (73 % vs. 41 %; p = 0.006). For PC2CP staining of ECM, no significant

Table 1 Main clinical data

Diagnosis	Enchondroma	Chondrosarcoma	*p* value
Number of cases	34 cases	43 cases	
		Grade I, 10	
		Grade II, 24	
		Grade III, 5	
		Dediff., 4	
Age (mean; [min-max])	37 years (6–63)	57 years (28–86)	$p < 0.0001$
Localization	Long bones, 62 % (21/34)	Long bones, 44 % (19/43)	$p < 0.005$
	Extremities, 35 % (12/34)	Extremities, 5 % (2/43)	
	Ribs, 3 % (1/34)	Ribs, 12 % (5/43)	
		Pelvis, 16 % (7/43)	
		Cranio-facial, 12 % (5/43)	
		Scapula, 2 % (1/43)	
		Spine, 5 % (2/43)	
		Soft tissues, 5 % (2/43)	
Sex ratio M/F	0.8	1.4	$p = 0.22$
5-year relapse rate	4 % (1/27)	43 % (12/28)	$p < 0.001$
		Grade I, 17 % (1/6)	
		Grade II, 36 % (5/14)	
		Grade III, 50 % (2/4)	
		Dediff., 50 % (2/4)	
5-year metastasis rate	0 % (0/27)	32 % (9/28)	$p < 0.005$
		Grade I, 17 % (1/6)	
		Grade II, 29 % (4/14)	
		Grade III, 50 % (2/4)	
		Dediff., 50 % (2/4)	
5-year overall survival	100 % (27/27)	82 % (23/28)	$p = 0.11$
Follow up length (median; [min–max])	12 months (0–94)	47 months (0–158)	$p < 0.0005$

M male, *F* female, *dediff.* dedifferentiated

differences were found between tumor and non-tumor groups, or between enchondromas and grade I CS (Table 2). In addition, the semiquantitative ECM score was higher in enchondromas than in CSs ($p = 0.02$) (Fig. 2), but not different between tumor and nontumor groups ($p > 0.5$). There was no correlation with recurrence-free survival or metastases.

The inter-assessor agreement was good for PC2CP ECM distribution (Kappa, 0.66; 95 % CI, 0.55–0.76) and intensity (Kappa, 0.64; 95 % CI, 0.52–0.75) scores, and very good for the semiquantitative PC2CP ECM score (ICC, 0.82; 95 % CI, 0.74–0.88) and cytoplasmic PC2CP immunoreactivity (Kappa, 0.82; 95 % CI, 0.58–1.00).

Diagnostic performances of PC1CP and PC2CP

The diagnostic performance of staining for PC1CP and PC2CP, separately and in combination, is reported in Table 3. To this end, diffuse ECM staining for PC1CP (staining of more than 50 % of ECM surface area) and lack of ECM staining for PC2CP (<5 % of ECM surface area) were taken as criteria for malignancy. Sensitivity and specificity of diffuse PC1CP staining or absence of PC2CP staining as single criteria was low. However, when combined as criterion for malignancy, the specificity was 94 % but with a sensitivity of only 20 % for grade I CS and 35 % for grade I and II CS. The highest sensitivity was obtained with diffuse PC1CP staining of absence of PC2CP staining as single criteria, reaching 82 % in grade I or grade II CS and 60 % for grade I CS but with a specificity of only 47 %.

Discussion

The ECM, with collagens as important component, has multiple and complex roles in tumor differentiation and progression [15]. The fibrillar collagens type I and II are essential for the formation of normal articular cartilage. Abnormal expression of these collagens plays a role in the biology of

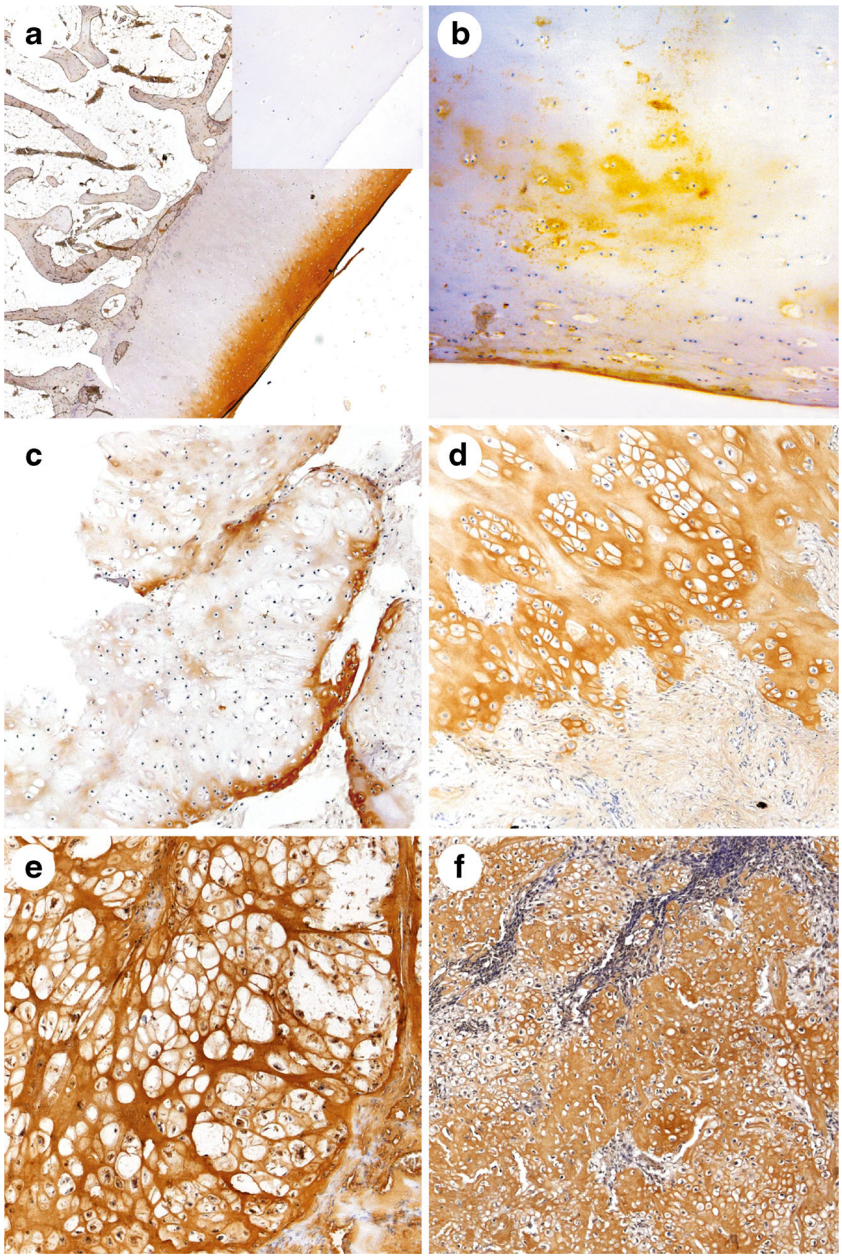

Fig 1 PC1CP. **a** Extracellular matrix (ECM) staining for PC1CP in superficial layers of normal cartilage (*insert* negative control—omission of primary antibody) (×25). **b** Weak staining extending to the intermediate layers in a case of arthritis. **c** Weak and focal ECM staining in an enchondroma ×100. **d** Moderate and partial ECM staining in a grade I chondrosarcoma ×100. **e**, **f** Diffuse and strong staining for PC1CP in a case of grade III chondrosarcoma (**e**) and dedifferentiated chondrosarcoma (**f**) (×100)

cartilaginous tumors [3]. Collagen I is mainly a heterotrimer with 2 Iα1 and 1 Iα2 chains; it can also exist as a homotrimer with 3 Iα1 chains. Aberrant homotrimeric collagen I expression has been associated with conditions such as osteoporosis, osteogenesis imperfecta, and cancer [16, 17]. In breast cancer cells, homotrimeric collagen I stimulates cell proliferation and migration [17, 18]. In CS, collagen I expression is associated with a dedifferentiated phenotype and increased cell proliferation [3, 9, 13, 19] and with differentiation to osteoblast-like cells [3]. The proportion of homotrimeric collagen I is reflected in the abundance of PC1CP, as this peptide is produced through cleavage of the COOH-terminal extremity of homotrimeric procollagen I. Collagen II is a homotrimer with three IIα1 chains and represents approximately 95 % of all collagen in normal cartilage. It has two alternative splice variants, COL2A expressed by chondroprogenitor cells, and COL2B expressed by mature chondrocytes. The level of collagen II is correlated to that of PC2CP (also termed chondrocalcin) as PC2CP is produced by cleavage of the COOH-terminal extremity of procollagen II, one of the most highly synthesized proteins in articular cartilage.

Using two-dimensional electrophoresis followed by MALDI-TOF mass spectrometry, we previously identified PC1CP and PC2CP as two proteins differentially expressed in malignant vs. benign cartilaginous tumors [14]. Increased PC1CP and decreased PC2CP expression in CS was subsequently confirmed using Western blots [14]. We also investigated in vitro the function of soluble or immobilized

Table 2 Results of immunohistochemistry

Group	Nontumoral (n = 11)	Enchondroma (n = 34)	Chondrosarcoma (n = 43)	p value
PC1CP				
ECM distribution				p = 0.03
- Score 1 (0–5 %)	45 %	29 %	16 %	
- Score 2 (6–25 %)	27 %	26 %	16 %	
- Score 3 (26–50 %)	18 %	26 %	23 %	
- Score 4 (>50 %)	0 %	18 %	44 %	
ECM intensity score				p = 0.07
- Score 0 (negative)	9 %	0 %	5 %	
- Score 1 (weak)	45 %	24 %	9 %	
- Score 2 (moderate)	36 %	35 %	44 %	
- Score 3 (strong)	9 %	41 %	42 %	
ECM global score (mean[min-max])	3.0 (0–6)	5.5 (1–12)	7.1 (0–12)	p = 0.01
Cytoplasm positivity	36 %	38 %	56 %	p = 0.34
Diffuse cytoplasm staining	0 %	12 %	33 %	p = 0.02
PC2CP				
ECM distribution				p = 0.001
- Score 1 (0–5 %)	82 %	41 %	67 %	
- Score 2 (6–25 %)	9 %	29 %	12 %	
- Score 3 (26–50 %)	9 %	18 %	9 %	
- Score 4 (>50 %)	0 %	12 %	12 %	
ECM intensity score				p = 0.001
- Score 0 (negative)	9 %	15 %	51 %	
- Score 1 (weak)	82 %	26 %	16 %	
- Score 2 (moderate)	9 %	12 %	12 %	
- Score 3 (strong)	0 %	12 %	9 %	
ECM global score (mean[min–max])	1.5 (0-6)	3.6 (0–12)	2.4 (0–12)	p = 0.07
Cytoplasm positivity	73 %	97 %	93 %	p = 0.03
Diffuse cytoplasm staining	27 %	62 %	67 %	p = 0.05

ECM extracellular matrix, *Dediff.* dedifferentiated (Kruskal-Wallis test for quantitative variables; chi-squared or Fisher's exact test for qualitative variables)

recombinant PC1CP and PC2CP in ECM. Immobilized PC2CP, but not PC1CP, induced apoptosis in primary chondrocytes and EAhy926 endothelial cells. In contrast, soluble PC1CP but not PC2CP induced migration of EAhy926 cells and increased expression of vascular endothelial growth factor (VEGF) and CXCR4 in chondrocytes. Soluble PC2CP also increased the expression of VEGF but along with a more pronounced effect on the expression of CXCR4 and matrix metalloproteinase 13 [14]. These data unequivocally support an oncogenic role of PC1CP and functional dualism of PC2CP in CS oncogenesis, depending on whether it is soluble or immobilized.

In the present study, we found significantly higher expression of PC1CP in ECM in tumor than in nontumor samples (p = 0.03) and in CSs than in enchondromas (p = 0.02). These results are in line with our earlier experimental data, showing oncogenic properties of PC1CP in the ECM. Staining was stronger in moderately differentiated or dedifferentiated areas, but not significantly correlated with histological grade or survival. Contrary to PC1CP, significantly lower ECM expression of PC2CP was found in CSs than in enchondromas, without significant difference between tumor and non-tumor groups. Taken together with earlier published results, our findings support an anti-oncogenic role of PC2CP. The fact that loss of function mutations of *COL2A1* are frequently found in CS [20] corroborates our hypothesis, since in CS decreased COL2 expression goes along with decreased expression of PC2CP.

The differential diagnosis between enchondroma and grade I or II CS can be challenging, most notably on biopsy specimens. Few markers have been found relevant and to date no marker has been validated to distinguish between benign and malignant well-differentiated tumors [13, 21]. We found distinctly different patterns of expression of PC1CP and PC2CP in ECM of CS and enchondroma. Regarding diagnostic performance of these markers, we found sensitivity and

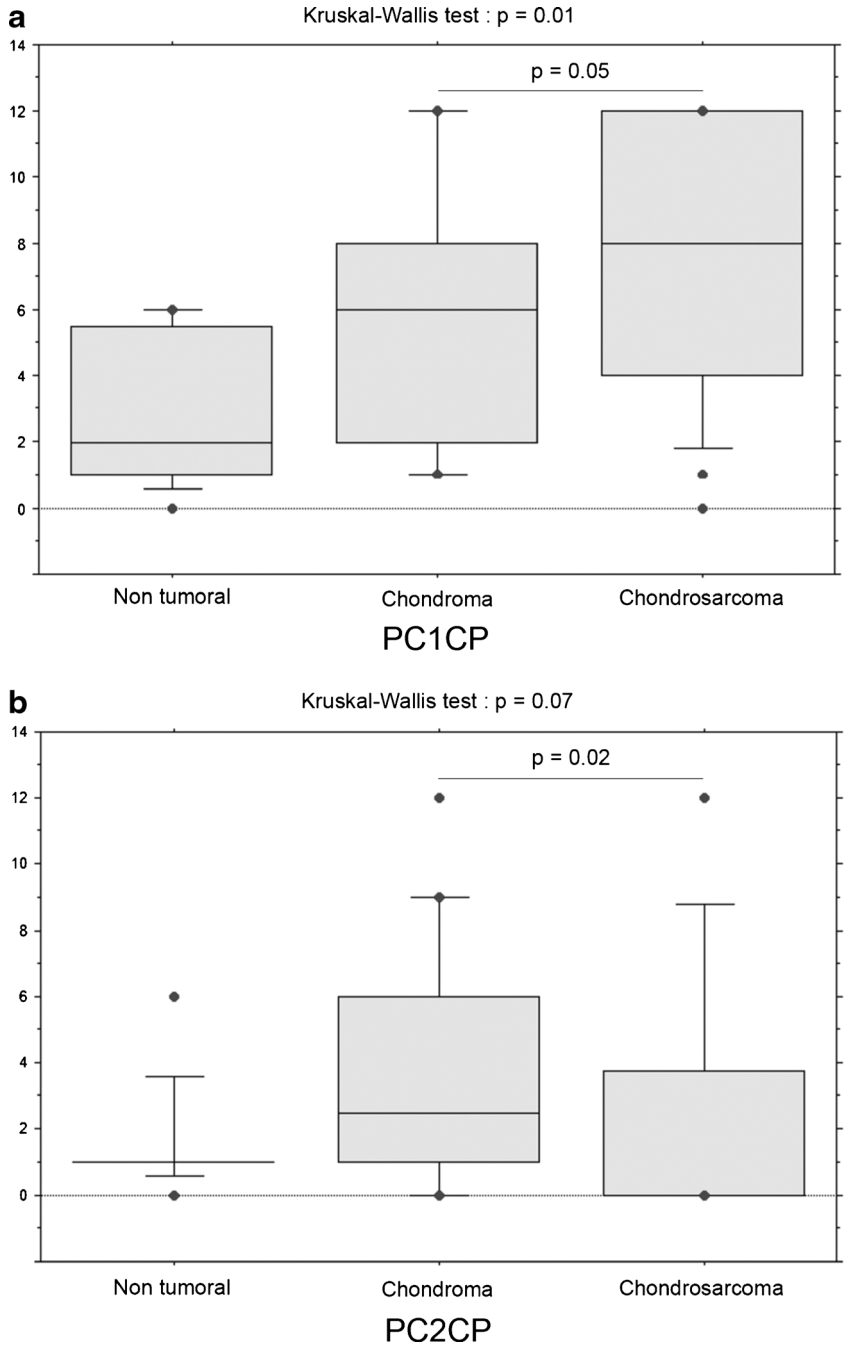

Fig. 2 Extracellular matrix global score for PC1CP and PC2CP: overexpression of PC1CP in chondrosarcomas vs. enchondromas ($p = 0.05$); lower expression of PC2CP in chondrosarcomas vs. enchondromas ($p = 0.02$). Boxplots (*center line* median, *box length* interquartile range, *whiskers* 10th–90th percentiles, *individual data points* unusual values). *Global comparison* Kruskal-Wallis test, *chondromas vs chondrosarcomas* Mann–Whitney test

specificity of these two markers too low to be clinically useful. When combined, however, diffuse PC1CP staining together with absence of PC2CP staining was highly specific (94 %) for CS. In a biopsy specimen with this diagnostic dilemma, this pattern of expression of PC1CP and PC2CP justifies suspicion of a diagnosis of CS and a curative surgical procedure. The drawback is low sensitivity (35 %). The single use of these markers as criterion for malignancy should not dismissed, as diffuse PC1CP staining or absence of PC2CP staining has a sensitivity as high as 83 % but low specificity. Even though this diagnostic performance is limited, it merits to be compared with that of classical histological parameters. The use of these markers in combination with others might also be considered.

In conclusion, we show that expression of PC1CP is higher and of PC2CP is lower in malignant cartilaginous tumors than in benign or non-neoplastic lesions. These results support an oncogenic role of PC1CP and an anti-oncogenic role of PC2CP in cartilaginous tumors. Further studies should be performed to evaluate the usefulness of these markers on biopsy specimens, alone or in association with other putative markers of malignancy.

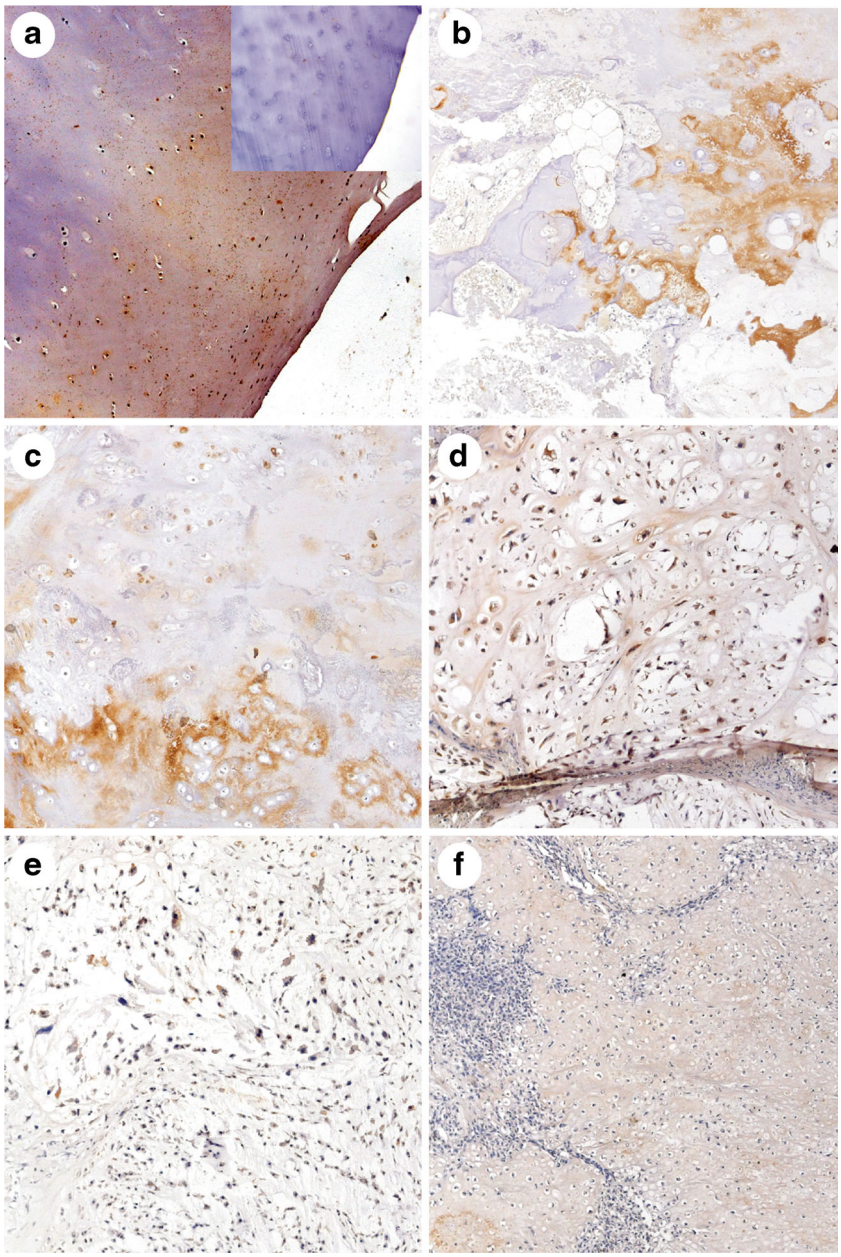

Fig. 3 PC2CP. **a** Mild extracellular matrix (ECM) staining for PC2CP in superficial and intermediate layers of normal cartilage (*insert* negative control) (×25). **b**, **c** Moderate ECM staining for PC2CP in a case of enchondroma **b** and grade I chondrosarcoma (**c**) (×100). **d** Weak staining in a grade II CS. **e** Negative ECM staining and diffuse cytoplasm staining for PC2CP in a grade III CS (×200). **f** Weak extracellular expression in the cartilaginous component of a dedifferentiated CS (×100)

Table 3 Diagnostic performances of PC1CP and PC2CP separately and in combination for the diagnosis of malignancy, facing a well-differentiated cartilaginous tumor

Malignant samples		Diffuse PC1CP (%)	PC2CP negativity (%)	Diffuse PC1CP and PC2CP negativity (%)	Diffuse PC1CP or PC2CP negativity (%)
Grade I CS	Sensitivity	30	50	20	60
	Specificity	82	59	94	47
Grades I and II CS	Sensitivity	50	68	35	82
	Specificity	82	59	94	47

Diffuse staining for PC1CP is defined by a staining of more than 50 % of extracellular matrix; PC2CP negativity, by a staining lesser than 5 %

CS chondrosarcoma

Acknowledgments The authors thank Elodie SPEYER (Epidemiology and Clinical Evaluation, CHU Nancy, France) for statistical analyses and all the team of the Department of Pathology (CHU Nancy) for technical support.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Fletcher CDM (2013) WHO classification of tumours of soft tissue and bone. IARC Press, Lyon
2. Eefting D, Schrage YM, Geirnaerdt MJ, Le Cessie S, Taminiau AH, Bovee JV, Hogendoorn PC (2009) Assessment of interobserver variability and histologic parameters to improve reliability in classification and grading of central cartilaginous tumors. Am J Surg Pathol 33:50–57. doi:10.1097/PAS.0b013e31817eec2b
3. Aigner T (2002) Towards a new understanding and classification of chondrogenic neoplasias of the skeleton–biochemistry and cell biology of chondrosarcoma and its variants. Virchows Arch 441:219–230. doi:10.1007/s00428-002-0641-x
4. Bovee JV, Hogendoorn PC (2010) Molecular pathology of sarcomas: concepts and clinical implications. Virchows Arch 456:193–199. doi:10.1007/s00428-009-0828-5
5. Bovee JV, Cleton-Jansen AM, Kuipers-Dijkshoorn NJ, van den Broek LJ, Taminiau AH, Cornelisse CJ, Hogendoorn PC (1999) Loss of heterozygosity and DNA ploidy point to a diverging genetic mechanism in the origin of peripheral and central chondrosarcoma. Genes Chromosom Cancer 26:237–246
6. Hallor KH, Staaf J, Bovee JV, Hogendoorn PC, Cleton-Jansen AM, Knuutila S, Savola S, Niini T, Brosjo O, Bauer HC, Vult von Steyern F, Jonsson K, Skorpil M, Mandahl N, Mertens F (2009) Genomic profiling of chondrosarcoma: chromosomal patterns in central and peripheral tumors. Clin Cancer Res 15:2685–2694. doi:10.1158/1078-0432.CCR-08-2330
7. Sandberg AA (2004) Genetics of chondrosarcoma and related tumors. Curr Opin Oncol 16:342–354
8. de Andrea CE, Wiweger MI, Bovee JV, Romeo S, Hogendoorn PC (2012) Peripheral chondrosarcoma progression is associated with increased type X collagen and vascularisation. Virchows Arch 460:95–102. doi:10.1007/s00428-011-1168-9
9. Aigner T, Dertinger S, Vornehm SI, Dudhia J, von der Mark K, Kirchner T (1997) Phenotypic diversity of neoplastic chondrocytes and extracellular matrix gene expression in cartilaginous neoplasms. Am J Pathol 150:2133–2141
10. Aigner T, Frischholz S, Dertinger S, Beier F, Girkontaite I, von der Mark K (1997) Type X collagen expression and hypertrophic differentiation in chondrogenic neoplasias. Histochem Cell Biol 107:435–440
11. Bovee JV, Cleton-Jansen AM, Wuyts W, Caethoven G, Taminiau AH, Bakker E, Van Hul W, Cornelisse CJ, Hogendoorn PC (1999) EXT-mutation analysis and loss of heterozygosity in sporadic and hereditary osteochondromas and secondary chondrosarcomas. Am J Hum Genet 65:689–698. doi:10.1086/302532
12. Schrage YM, Hameetman L, Szuhai K, Cleton-Jansen AM, Taminiau AH, Hogendoorn PC, Bovee JV (2009) Aberrant heparan sulfate proteoglycan localization, despite normal exostosin, in central chondrosarcoma. Am J Pathol 174:979–988. doi:10.2353/ajpath.2009.080623
13. Aigner T, Muller S, Neureiter D, Illstrup DM, Kirchner T, Bjornsson J (2002) Prognostic relevance of cell biologic and biochemical features in conventional chondrosarcomas. Cancer 94:2273–2281. doi:10.1002/cncr.10461
14. Vincourt JB, Etienne S, Cottet J, Delaunay C, Malanda CB, Lionneton F, Sirveaux F, Netter P, Plenat F, Mainard D, Vignaud JM, Magdalou J (2010) C-propeptides of procollagens I alpha 1 and II that differentially accumulate in enchondromas versus chondrosarcomas regulate tumor cell survival and migration. Cancer Res 70:4739–4748. doi:10.1158/0008-5472.CAN-10-0046
15. Fang M, Yuan J, Peng C, Li Y (2014) Collagen as a double-edged sword in tumor progression. Tumour Biol 35:2871–2882. doi:10.1007/s13277-013-1511-7
16. Pace JM, Wiese M, Drenguis AS, Kuznetsova N, Leikin S, Schwarze U, Chen D, Mooney SH, Unger S, Byers PH (2008) Defective C-propeptides of the proalpha2(I) chain of type I procollagen impede molecular assembly and result in osteogenesis imperfecta. J Biol Chem 283:16061–16067. doi:10.1074/jbc.M801982200
17. Han S, Makareeva E, Kuznetsova NV, DeRidder AM, Sutter MB, Losert W, Phillips CL, Visse R, Nagase H, Leikin S (2010) Molecular mechanism of type I collagen homotrimer resistance to mammalian collagenases. J Biol Chem 285:22276–22281. doi:10.1074/jbc.M110.102079
18. Makareeva E, Han S, Vera JC, Sackett DL, Holmbeck K, Phillips CL, Visse R, Nagase H, Leikin S (2010) Carcinomas contain a matrix metalloproteinase-resistant isoform of type I collagen exerting selective support to invasion. Cancer Res 70:4366–4374. doi:10.1158/0008-5472.CAN-09-4057
19. Dornauer K, Soder S, Inwards CY, Bovee JV, Aigner T (2010) Matrix biochemistry and cell biology of dedifferentiated chondrosarcomas. Pathol Int 60:365–372. doi:10.1111/j.1440-1827.2010.02530.x
20. Tarpey PS, Behjati S, Cooke SL, Van Loo P, Wedge DC, Pillay N, Marshall J, O'Meara S, Davies H, Nik-Zainal S, Beare D, Butler A, Gamble J, Hardy C, Hinton J, Jia MM, Jayakumar A, Jones D, Latimer C, Maddison M, Martin S, McLaren S, Menzies A, Mudie L, Raine K, Teague JW, Tubio JM, Halai D, Tirabosco R, Amary F, Campbell PJ, Stratton MR, Flanagan AM, Futreal PA (2013) Frequent mutation of the major cartilage collagen gene COL2A1 in chondrosarcoma. Nat Genet 45:923–926. doi:10.1038/ng.2668
21. Helfenstein A, Frahm SO, Krams M, Drescher W, Parwaresch R, Hassenpflug J (2004) Minichromosome maintenance protein (MCM6) in low-grade chondrosarcoma: distinction from enchondroma and identification of progressive tumors. Am J Clin Pathol 122:912–918. doi:10.1309/G638-TKNN-G2CJ-UXWL