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Periosteal chondrosarcoma: a histopathological and molecular analysis of a rare chondrosarcoma subtype

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Periosteal chondrosarcoma: a histopathological and molecular analysis of a rare chondrosarcoma subtype

Aims: Periosteal chondrosarcoma is a rare, malignant cartilage-forming neoplasm originating from the periosteal surface of bone. We collected 38 cases from the archives of the Netherlands Committee on Bone Tumours, with the aim of studying histological features and evaluating the involvement of isocitrate dehydrogenase 1 (IDH1), EXT, Wnt/ β -catenin, the pRB pathway (CDK4 and p16), and the TP53 pathway (p53 and MDM2).

Methods and results: Histology showed a moderately cellular matrix with mucoid–myxoid changes and, in 42% of cases, formation of a neocortex. Occasional intramedullary extension (26%) and subsequent host bone entrapment (40%) were seen. Histological grading revealed grade 1 (53%) and grade 2 (45%). The

EXT1 protein was normally expressed, and mutations in *IDH1* were observed in only 15% of cases. pRb signalling was deregulated by loss of p16 expression in 50% of cases, and Wnt signalling was lost in 89%. No alterations were found in CDK4, p53, or MDM2.

Conclusions: We report the first large histological and molecular study on periosteal chondrosarcoma showing that histopathological examination and molecular aberrations do not predict prognosis. Although the mutation frequency of *IDH1* was low, we confirm the supposed relationship with central chondrosarcoma. Moreover, we identify loss of canonical Wnt signalling and deregulation of pRb signalling as possible events contributing to its histogenesis.

Keywords: bone tumour, chondrosarcoma, IDH1, periosteal chondrosarcoma

Introduction

Chondrosarcomas constitute a heterogeneous group of cartilaginous matrix-producing neoplasms with diverse morphological features and clinical behaviours.¹ After osteosarcomas, they are the most common primary malignant tumours of bone.² Conventional chondrosarcomas are classified according to their site of origin in the bone.¹ Seventy-five per cent arise in the medulla, and are termed conven-

tional central chondrosarcomas. Approximately 10% of conventional chondrosarcomas arise secondarily in a pre-existing benign osteochondroma at the surface of the bone, and are designated as secondary peripheral chondrosarcoma.¹ In addition to conventional chondrosarcomas, some rare chondrosarcoma subtypes are recognized, including dedifferentiated (~10%), clear cell (<2%) and mesenchymal (<2%) chondrosarcomas.¹ In addition, periosteal chondrosarcoma, previously known as juxtacortical chondrosarcoma, was first described by Lichtenstein in 1955, and accounts for <1% of chondrosarcomas. It is defined by the World Health Organization (WHO) as 'a malignant hyaline cartilage neoplasm, which

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occurs on the surface of bone and originates from the periosteum'.¹

So far, radiological and histological diagnostic criteria for periosteal chondrosarcoma have been based on small series and case reports. Moreover, its molecular background was largely unknown until mutations in isocitrate dehydrogenase 1 (IDH1), which is involved in the tricarboxylic acid cycle, were reported in central and periosteal chondrosarcomas.^{3,4} However, only 10 periosteal chondrosarcomas have been reported so far, six of which carried a hotspot mutation in *IDH1*.^{3–5} In contrast, peripheral cartilaginous tumours are caused by inactivating germline mutations or somatic homozygous deletion of the genes encoding exostosis (multiple)-1 (*EXT1*) or exostosis (multiple)-2 (*EXT2*), which catalyse the biosynthesis of heparan sulphate proteoglycans involved in signalling pathways, especially in the normal growth plate.^{6–8} The involvement of EXTs in periosteal chondrosarcoma has not been investigated so far. Interestingly, loss of β -catenin, an important player in the Wnt signalling pathway, which is essential for bone homeostasis, was shown to induce multifocal periosteal chondroma-like masses in mice.⁹ In conventional chondrosarcoma, the pRB and TP53 pathways are involved in progression towards higher histological grade, but their significance in periosteal chondrosarcoma is unknown.¹⁰

As diagnostic criteria are based on small series and case reports, and larger series are so far lacking, we collected 38 periosteal chondrosarcomas from the archives of the Netherlands Committee on Bone Tumours (NCBT) and evaluated their histological features. Additionally, we evaluated the involvement of the *IDH* and *EXT* genes, as well as the Wnt- β -catenin, pRB (CDK4 and p16), and TP53 (p53 and MDM2) pathways.

Materials and methods

SELECTION CRITERIA

Forty-eight cases of cartilaginous neoplasm previously diagnosed as periosteal chondrosarcoma were collected. Forty-four cases were retrieved from the archives of the NCBT; diagnosis was made by joint assessment by radiologists and pathologists. The archives of the NCBT contain clinical, radiological and histological material of ~17 000 bone tumours and tumour-like lesions of bone, collected since 1953. Four cases were added from the electronic patient database of the Leiden University Medical Centre. All material was handled according to the

ethical guidelines described in the Code for Proper Secondary Use of Human Tissue in The Netherlands.

All cases were reviewed by two experienced bone tumour pathologists (P.C.W.H. and J.V.M.G.B.), and the diagnostic criteria of the 2013 WHO classification¹ were used. Lesions additionally had to meet at least one of the following criteria: size of ≥ 50 mm, cortical invasion, or soft tissue extension. Radiographs were reviewed by one experienced bone tumour radiologist (H.K.).

PATIENT DATA

Detailed clinical data, including age, duration of complaints, symptoms, and treatment, were collected for each case from the files, and have been recently published.¹¹ Follow-up data were available for 30 patients, and were updated by consulting patient records up to their discharge from postoperative examinations.

HISTOLOGY

Haematoxylin and eosin (H&E)-stained sections from resections, excisions or biopsies were available for 38 cases. Architectural and cytological parameters¹² were systematically scored by an experienced bone tumour pathologist (J.V.M.G.B.), including matrix (percentage of hyaline and mucoid–myxoid differentiation), cellularity (low, moderate, and high), cell distribution, secondary periosteal bone formation, calcification, encasement, cortical extension, host bone entrapment (assessed when medullary extension was present), nuclear polymorphism, binucleated cells [$\leq 2 / > 2$ per 10 high-power fields (HPFs)], mitosis (present or absent, per 10 HPFs), and nuclei and chromatin (percentage of condensed nuclei and open chromatin differentiation). In addition, we evaluated endochondral ossification, formation of a neocortex, medullary extension, and visible nucleoli. Cases were also graded according to the grading system for chondrosarcomas postulated by Evans *et al.*,¹³ which was adopted by the WHO in 2013.¹

IMMUNOHISTOCHEMISTRY

Formalin-fixed paraffin-embedded material after decalcification was available for 24 cases. Immunohistochemistry was used to study IDH1 R132H, β -catenin, *EXT1*, p16, CDK4, p53, and MDM2. For all immunohistochemical staining, negative controls were included with 1% phosphate-buffered saline/bovine

serum albumin instead of the first antibody. The antibodies used, antibody concentrations, antigen retrieval, positive controls and other antibody specifications are described in Table S1. Slides were semiquantitatively scored for cytoplasmic and/or nuclear staining (0, negative; 1, weak; 2, moderate; 3, strong) and for the percentage of positive cells (0, 0%; 1, 1–24%; 2, 25–49%; 3, 50–74%; 4, 75–100%) independently by two observers (A.H.G.C. and J.V.M.G.B.), who later reached consensus on each score for which there was a discrepancy. Scores were added, and cut-off levels for rendering a case positive for statistical analysis were applied (sum of score of ≥ 2 for IDH1 R132H, EXT1, CDK4, p16, and MDM2; sum of score of ≥ 3 for p53 and β -catenin). For p16, endothelial cells served as an internal positive control to determine whether p16-negative tumours were truly negative or negative because of decalcification. Samples without an internal positive control were excluded from the analysis for p16. For β -catenin, only nuclear staining in at least 25% of the tumour cells¹⁴ was regarded as positive in the final analysis. Cases with only cytoplasmic staining or no staining at all were regarded as negative.

GENOMIC ANALYSIS

Genomic DNA was isolated from 23 cases by the use of whole slides or microdissection to obtain at least 80% tumour cells. One case was omitted because of insufficient viable tumour cells, on the basis of H&E-stained slides. DNA was isolated as described previously.¹⁵ Conventional Sanger sequencing of exon 4 of *IDH1* and exon 4 of *IDH2*, as well as a more sensitive hydrolysis probe assay to specifically detect R132C and R132H *IDH1* mutations, were performed as described previously^{4,16,17} (Table S2). Fluorescence *in-situ* hybridization (FISH) was performed for *MDM2* amplification in two cases showing nuclear *MDM2* expression with the Histology FISH Accessory Kit (DakoCytomation, Glostrup, Denmark), according to the manufacturer's protocol.

STATISTICAL ANALYSIS

Comparison of means between groups was performed with the Mann–Whitney test. Correlations between the studied variables were analysed with the Pearson chi-square test or Fisher's exact test as appropriate. A *P*-value of <0.05 was considered to be significant. All statistical analyses were performed with IBM SPSS STATISTICS 20 (IBM, Armonk, NY, USA).

Results

CASE SERIES

The diagnosis of periosteal chondrosarcoma was confirmed in 44 of 48 cases by joint assessment by the radiologist and pathologists. Four cases were excluded because of a diagnosis that was different from the original one, i.e. three periosteal osteosarcomas and one osteochondroma. An additional six cases were excluded because either radiological or histological material could not be used for revision. Thirty-eight cases remained for use in this study, originating from 22 different contributing institutions.

The clinical and radiological data of our cohort have been described elsewhere by others.¹¹ In brief, they were as follows. There was a slight male predominance (22 males versus 16 females). The median age at diagnosis was 28 years (range 10–76 years). The median diameter of the tumour was 40 mm (range 10–125 mm). Most lesions occurred in the metaphysis or diaphysis of the long tubular bones (87%, $n = 33$), with the proximal humerus (33%) and distal femur (33%) being most frequently affected.

Radiologically (Figure 1A), periosteal chondrosarcoma presented as osteolytic lesions with lobulated morphology, a thinned underlying cortex, and a periosteal reaction, and were covered, at least in part, by a peripheral calcified shell. Lesions <50 mm radiologically showed cortical invasion and/or soft tissue extension. Macroscopic examination typically showed tumour lobules extending into the surrounding soft tissue (Figure 1B,C) and infiltration of the underlying cortical bone (Figure 1D). Nine patients received local treatment (eight intralesional excisions and one marginal excision), and 29 patients received extended treatment consisting of wide *en-bloc* resection or amputation. The median follow-up for 30 patients for whom data were available was 2.5 years (range 0.17–23 years). Residual tumour was found in nine patients. Two of 30 patients (6.7%) developed metastases after initial intralesional excision, one after 2 years of initial treatment and the second after 17 years. One of these also developed local recurrence. Both patients with metastasis had grade 2 tumours; the tumour sizes were 30 and 42 mm.

HISTOLOGICAL ANALYSIS

On low-power microscopy (Figure 2A), periosteal chondrosarcomas presented as a lobulated cartilage mass, in which cells were irregularly distributed in

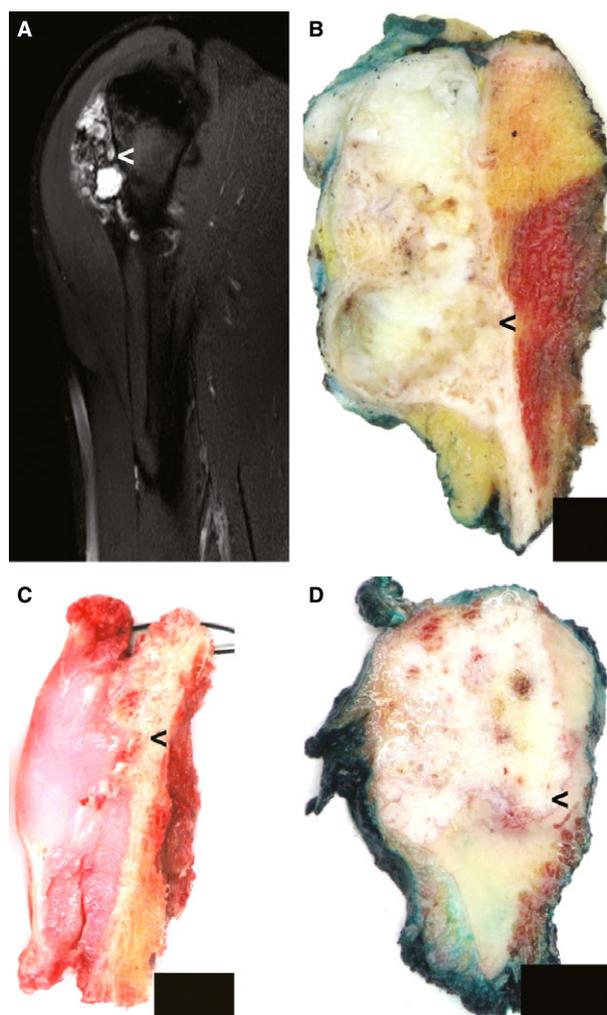


Figure 1. A, In the right proximal humeral metaphysis, there is a periosteally located large lobulated cartilage tumour (white arrowhead) with a maximum diameter of 55 mm, extending into the soft tissue (T1-weighted gadolinium magnetic resonance image). B, Macroscopic image of the periosteal cartilage tumour shown in (A). The black arrowhead indicates erosion of the cortex. C, D, Macroscopic image showing a periosteal lobulated cartilage tumour with erosion (C) and invasion (D) of cortical bone (black arrowhead). B, C, D, Black box from left to right = 10 mm.

87% (33/38) and embedded within a predominantly mucoid–myxoid matrix in >60% (Figure 2B,C). Lesions were moderately to highly cellular in 92% (35/38), with low to moderate nuclear pleomorphism in 90% (34/38), with readily seen binucleated cells in 66% (25/38), and with condensed nuclei in 61% (23/38) (Figure 2D). Nucleoli were seen in 55% (21/38), and mitoses were seen in 42% (16/38). A small majority (53%, 20/38) of cases showed grade 1 morphology, and 45% (17/38) and 2% (1/38) showed grade 2 and grade 3 morphology, respectively.¹³ Secondary bone formation and calcification were seen in

71% (27/38) and 55% (21/38), respectively. Cortical invasion (Figure 2E) was seen in 69% (26/38), with host bone entrapment in 40% (four of 10 cases in which this could be assessed). Neocortex formation, the formation of a layer of compact mature bone surrounding the tumour (Figure 2F), is a feature uncommon to other subtypes of chondrosarcoma, and was seen in 42% (16/38). Endochondral ossification and encasement were not often seen (18% and 21%, respectively). None of the histological features, including histological grading, was associated with the development of metastasis or local recurrence (data not shown).

MOLECULAR ANALYSIS

Isocitrate dehydrogenase mutation analysis was successful in 12 of 23 cases, one of which harboured the *IDH1* R132C mutation, as detected both by conventional Sanger sequencing and by hydrolysis probe assay (Table S3). Immunohistochemistry for *IDH1* R132H revealed one additional mutation in a case for which DNA extraction failed (Figure 3A; Table S4). Cytoplasmic expression of *EXT1* was observed in all cases, suggesting normal function of *EXT* and an absence of *EXT*-inactivating mutations (Figure 3B). Nuclear β -catenin staining was found in only two of 18 cases, with 89% showing loss of β -catenin expression (Figure 3C; Table S4). p16 expression was lost in 50% (8/16) (Figure 3D; Table S4). Nuclear expression of *CDK4* was absent in all cases (Table S4). Two cases showed nuclear expression of *MDM2* (Table S4); however, gene amplification was absent, as demonstrated by FISH analysis (data not shown). p53 showed wild-type staining (Table S4), and mutation analysis for *TP53* in five cases with slightly increased nuclear expression of p53 revealed no mutations (data not shown). None of the molecular features was associated with histological grade, the development of metastasis, or local recurrence (data not shown).

Discussion

A PubMed search of the English-language literature for 'periosteal chondrosarcoma' and 'juxtacortical chondrosarcoma' resulted in available full-text articles with, in total, 109 cases from 1955 until 2013, including a few small series containing up to 24 cases^{18–24} and case reports.^{25–41} Using the archives of the NCBT, we collected a relatively large series of 38 periosteal chondrosarcomas, for which clinicoradiological features have been described elsewhere by

Figure 2. A, Periosteal chondrosarcoma with lobulated architecture. B, C, Tumour cells in a mucoid–myxoid changed matrix. D, Binucleated tumour cells and condensed nuclei. E, Cortical invasion of the tumour (black arrowhead). F, Formation of neocortex surrounding the tumour (black arrowheads).

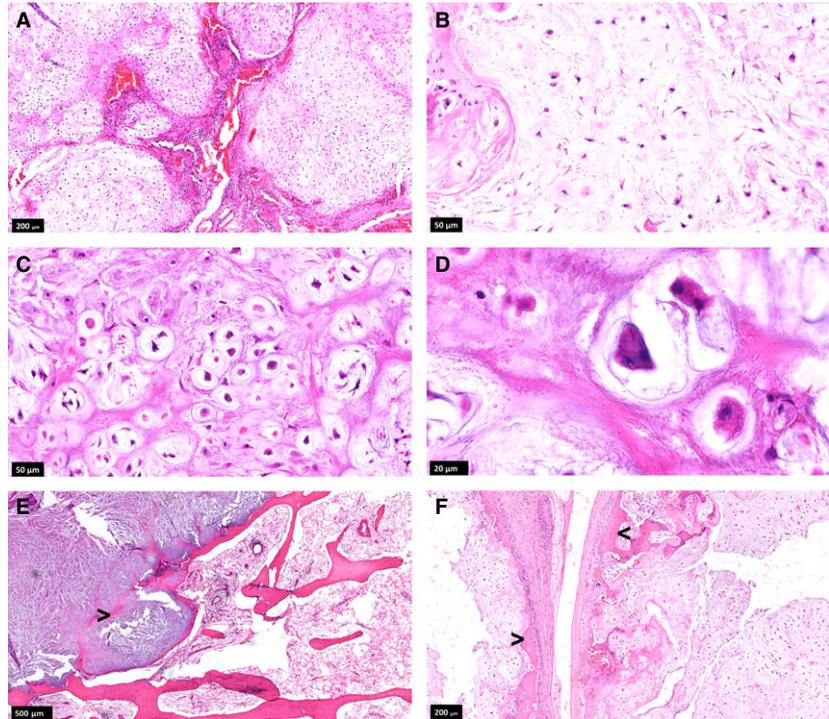
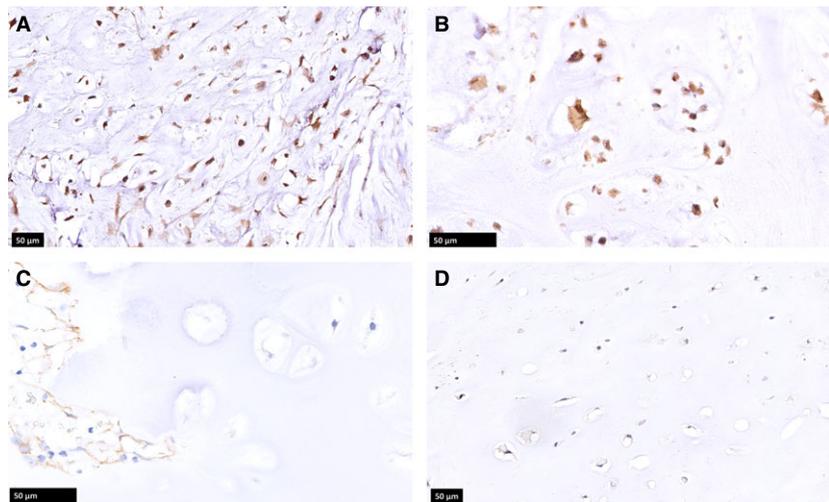


Figure 3. Immunohistochemistry showing IDH1 R132H expression in tumour cells (A), EXT1 cytoplasmic expression in tumour cells (B), loss of nuclear β -catenin expression in tumour cells (C), and loss of nuclear p16 expression in tumour cells (D).



others.¹¹ Here, we report their histological and molecular features.

Periosteal chondrosarcoma is a rare chondrosarcoma subtype that predominantly affects the long tubular bones, especially the distal femur, followed by the humerus. It has a peak in the third decade, with a wide age range (9–79 years in all cases retrieved from the literature), and a male predominance (including our own series: in total, 97 males and 50 female patients).

The main differential diagnosis includes periosteal chondroma, for which local excision is sufficient.⁴²

Periosteal chondrosarcoma and periosteal chondroma can show overlapping radiological and histological features. A size exceeding 50 mm and/or invasion of the underlying cortical bone have been reported as criteria for diagnosing periosteal chondrosarcoma.^{1,19,43} Periosteal chondrosarcomas smaller than 50 mm have been reported;^{24–26,31,34,35,39,41} in these cases, the presence of cortical invasion and/or soft tissue extension favours malignancy. Histologically, we observed cortical invasion in the majority of our cases, favouring periosteal chondrosarcoma. In addition to invasion of the underlying cortex, periosteal chondrosarcomas frequently

show formation of a neocortex, soft tissue extension, increased cellularity, multinucleated cells, mitotic figures, and more prominent cytonuclear atypia manifested by nuclear pleomorphism and hyperchromasia, as also reported previously.^{24,44,45}

Periosteal chondrosarcomas should also be distinguished from secondary peripheral chondrosarcomas. In the latter, the medulla of the underlying bone is continuous with that of the stalk of the lesion, whereas in periosteal chondrosarcoma the underlying cortex can usually still be recognized. Moreover, periosteal osteosarcoma, which is often of the chondroblastic subtype, should be considered in the differential diagnosis, as it has a much worse prognosis.^{19,46} Direct deposition of osteoid by tumour cells is, by definition, absent in periosteal chondrosarcoma,^{1,28,39,47} and the cartilaginous areas in periosteal osteosarcomas show more severe cytonuclear pleomorphism than those in periosteal chondrosarcoma.

Periosteal chondrosarcoma has a relatively low metastatic rate [5% in the current series, as compared with 10 of 82 patients (12.2%) retrieved from the literature], and metastases especially involve the lungs,^{19,24,36} and rarely the lymph nodes.²⁷ As metastases have been described 18 years after initial treatment,²⁴ periosteal chondrosarcoma may seem to have a prolonged clinical course, necessitating long-term follow-up. None of the histological or molecular features that we systematically analysed were associated with the occurrence of metastases. Importantly, histological grading was also not predictive of outcome. This suggests that, unlike in previous reports, in our series histological grading of periosteal chondrosarcoma is of no prognostic value.^{11,24,27} However, it must be noted that the median follow-up period of 2.5 years in this series is relatively short, and that only two patients developed metastases, hampering meaningful correlative studies.

As molecular studies on larger series of periosteal chondrosarcomas have not been reported, we evaluated the involvement of the *IDH* and *EXT* genes, which are known to be involved in conventional central and peripheral cartilaginous tumours, respectively. Whereas *IDH* mutations have been reported in ~50% of conventional central chondrosarcomas,^{3,4} only a small subset (15%) of the periosteal chondrosarcomas in our series were positive. This is lower than the prevalence (in total, six of 10) of these mutations previously reported.^{3–5} Mutations in codon 100 of *IDH1* have been described in gliomas with no other mutations in *IDH1* or *IDH2*;¹⁶ however, we also did not detect *IDH1* R100Q mutations in our series. Inactivation of *EXT1* or *EXT2* underlies the

development of osteochondroma, causing a subset of tumour cells to be negative for the *EXT* protein.⁴⁸ As the protein was normally expressed, periosteal chondrosarcoma seems to be unrelated to osteochondroma and secondary peripheral chondrosarcoma. Loss of β -catenin, an important player in the Wnt signalling pathway, has been shown to induce multifocal periosteal chondroma-like masses in mice.⁹ Indeed, nuclear expression of β -catenin was lacking in the vast majority of human periosteal chondrosarcomas. We previously demonstrated nuclear β -catenin staining in 53% of osteochondromas and 19% of grade 1 peripheral chondrosarcomas, whereas it was absent in high-grade peripheral chondrosarcomas.⁴⁹ Among central tumours, 17% of enchondromas were positive, whereas 47% of atypical cartilaginous tumour/grade 1 chondrosarcomas were positive, 29% of grade 2 chondrosarcomas were positive, and 11% of grade 3 chondrosarcomas were positive. Thus, canonical Wnt signalling seems to decrease with increasing histological grade in conventional chondrosarcoma, whereas it is generally low in periosteal chondrosarcoma.

In conventional chondrosarcoma, the pRb and TP53 pathways are involved in progression towards higher histological grade.¹⁰ In our series of periosteal chondrosarcomas, we detected loss of protein expression of p16 in 50% of the cases, whereas no alterations were found in CDK4, TP53, or MDM2. Loss of the cell cycle regulator CDKN2A/p16/INK4A was previously indicated to play an important role in central chondrosarcoma progression.^{50,51} Here, we demonstrate that the pRb pathway is also deregulated by loss of p16 expression in half of the periosteal chondrosarcomas. Currently, there are no data available on the pRb or TP53 pathways in periosteal chondromas to compare with our series of periosteal chondrosarcomas.

In summary, we report the first relatively large histological and molecular study on periosteal chondrosarcoma showing that histopathological examination, including grade and molecular aberrations, does not predict prognosis. Although the mutation frequency of *IDH1* was low, we have confirmed the supposed relationship with central chondrosarcoma. Moreover, we have identified loss of canonical Wnt signalling and deregulation of pRb signalling by loss of p16 expression as possible events contributing to its histogenesis.

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Author contributions

Arjen H. G. Cleven: primary writer of the manuscript, and data analysis. Evita Zwartkruis: data analysis and laboratory activities. Pancras C. W. Hogendoorn: initiator of the study, and co-writer. Herman M. J. A. Kroon: reviewed all radiographs of the study cases. Inge Briaire-de Bruijn: technical laboratory assistance. Judith V. M. G. Bovée: initiator of the study, primary supervisor of the project, and co-writer.

Conflict of interest

All authors state that they have no conflicts of interest.

References

- Hogendoorn PCW, Bovée JVMG, Nielsen GP. Chondrosarcoma (grades I–III), including primary and secondary variants and periosteal chondrosarcoma. In Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F eds. *World Health Organization classification of tumours. Pathology and genetics of tumours of soft tissue and bone*. 4th ed. Lyon: IARC Press, 2013; 264–268.
- Dorfman HD, Czerniak B. Bone cancers. *Cancer* 1995; **75**: 203–210.
- Amary MF, Bacsi K, Maggiani F *et al*. IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. *J. Pathol.* 2011; **224**: 334–343.
- Pansuriya TC, van ER, d'Adamo P *et al*. Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. *Nat. Genet.* 2011; **43**: 1256–1261.
- Carter JM, Voss JS, Kipp BR, Wenger DE, Inwards CY. Isocitrate dehydrogenase (IDH1/IDH2) mutation types and frequency in periosteal cartilaginous tumors. 2013.
- Fuerer C, Habib SJ, Nusse R. A study on the interactions between heparan sulfate proteoglycans and Wnt proteins. *Dev. Dyn.* 2010; **239**: 184–190.
- Hameetman L, David G, Yavas A *et al*. Decreased EXT expression and intracellular accumulation of HSPG in osteochondromas and peripheral chondrosarcomas. *J. Pathol.* 2007; **211**: 399–409.
- Schrage YM, Hameetman L, Szuhai K *et al*. Aberrant heparan sulfate proteoglycan localization, despite normal exostosin, in central chondrosarcoma. *Am. J. Pathol.* 2009; **174**: 979–988.
- Cantley L, Saunders C, Guttenberg M *et al*. Loss of β -catenin induces multifocal periosteal chondroma-like masses in mice. *Am. J. Pathol.* 2013; **182**: 917–927.
- Bovee JVMG, Hogendoorn PCW, Wunder JS, Alman BA. Cartilage tumours and bone development: molecular pathology and possible therapeutic targets. *Nat. Rev. Cancer* 2010; **10**: 481–488.
- Goedhart LM, Ploegmakers JJ, Kroon HM, Zwartkruis EC, Jutte PC. The presentation, treatment and outcome of periosteal chondrosarcoma in the Netherlands. *Bone Joint J.* 2014; **96-B**: 823–828.
- Eefting D, Schrage YM, Geirnaerd MJ *et al*. Assessment of interobserver variability and histologic parameters to improve reliability in classification and grading of central cartilaginous tumors. *Am. J. Surg. Pathol.* 2009; **33**: 50–57.
- Evans HL, Ayala AG, Romsdahl MM. Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. *Cancer* 1977; **40**: 818–831.
- Ng TL, Gown AM, Barry TS *et al*. Nuclear beta-catenin in mesenchymal tumors. *Mod. Pathol.* 2005; **18**: 68–74.
- de Jong D, Verbeke SL, Meijer D, Hogendoorn PC, Bovee JV, Szuhai K. Opening the archives for state of the art tumour genetic research: sample processing for array-CGH using decalcified, formalin-fixed, paraffin-embedded tissue-derived DNA samples. *BMC Res. Notes* 2011; **4**: 1–11.
- Pusch S, Sahm F, Meyer J, Mittelbronn M, Hartmann C, von Deimling A. Glioma IDH1 mutation patterns off the beaten track. *Neuropathol. Appl. Neurobiol.* 2011; **37**: 428–430.
- van Oosterwijk JG, de Jong D, van Ruler MA *et al*. Three new chondrosarcoma cell lines: one grade III conventional central chondrosarcoma and two dedifferentiated chondrosarcomas of bone. *BMC Cancer* 2012; **12**: 375–385.
- Bertoni F, Boriani S, Laus M, Campanacci M. Periosteal chondrosarcoma and periosteal osteosarcoma. Two distinct entities. *J. Bone Joint Surg. Br.* 1982; **64**: 370–376.
- Nojima T, Unni KK, McLeod RA, Pritchard DJ. Periosteal chondroma and periosteal chondrosarcoma. *Am. J. Surg. Pathol.* 1985; **9**: 666–677.
- Schajowicz F. Juxtacortical chondrosarcoma. *J. Bone Joint Surg. Br.* 1977; **59-Bp**: 473–480.
- Vanel D, De Paolis M, Monti C, Mercuri M, Picci P. Radiological features of 24 periosteal chondrosarcomas. *Skeletal Radiol.* 2001; **30**: 208–212.
- Chaabane S, Bouaziz MC, Drissi C, Abid L, Ladeb MF. Periosteal chondrosarcoma. *Am. J. Roentgenol.* 2009; **192**: W1–W6.
- Robinson P, White LM, Sundaram M *et al*. Periosteal chondroid tumors: radiologic evaluation with pathologic correlation. *Am. J. Roentgenol.* 2001; **177**: 1183–1188.
- Papagelopoulos PJ, Galanis EC, Mavrogenis AF *et al*. Survivorship analysis in patients with periosteal chondrosarcoma. *Clin. Orthop. Relat. Res.* 2006; **448**: 199–207.
- Jokl P, Albright JA, Goodman AH. Juxtacortical chondrosarcoma of the hand. *J. Bone Joint Surg. Am.* 1971; **53**: 1370–1376.
- Lichtenstein L. Tumors of periosteal origin. *Cancer* 1955; **8**: 1060–1069.
- Matsumoto K, Hukuda S, Ishizawa M, Saruhashi Y, Okabe H, Asano Y. Parosteal (juxtacortical) chondrosarcoma of the humerus associated with regional lymph node metastasis: a case report. *Clin. Orthop.* 1993; **290**: 168–173.
- Putti TC, Kahn LB, Aprin H. Periosteal chondrosarcoma—a case report and review of the literature. *Arch. Pathol. Lab. Med.* 1997; **121**: 70–74.
- Ariel IM, Jacobs PA. Juxtacortical chondrosarcoma. The nature of fibrosarcoma recurrence necessitating interscapulothoracic amputation. *Am. J. Surg.* 1964; **108**: 874–879.
- Chano T, Matsumoto K, Ishizawa M, Morimoto S, Hukuda S, Okabe H. Periosteal osteosarcoma and parosteal chondrosarcoma evaluated by double immunohistochemical staining. Report of 2 cases. *Acta Orthop. Scand.* 1994; **65**: 355–358.
- Cooper RR. Juxtacortical chondrosarcoma: a case report. *J. Bone Joint Surg. Am.* 1965; **47**: 524–528.
- Costa EMC, Lucas BL, Silva MR, Vilarinho RH, De Faria PR, Loyola AM. Mandibular periosteal (juxtacortical) chondrosarcoma. *Braz. Dent. J.* 2009; **20**: 428–433.

33. Efremidou EI, Oikonomou A, Pavlidou E, Drosos G, Koutsopoulos A, Liratzopoulos N. Juxtacortical clavicular chondrosarcoma: diagnostic dilemmas: case report and review of literature. *Clin. Med. Insights Oncol.* 2013; **7**: 13–19.
34. Haliloglu N, Sahin G, Ekin C. Periosteal chondrosarcoma of the foot: a case report. *J. Foot Ankle Surg.* 2010; **49**: 488.e1–488.e4.
35. Kavitha KP, Savithri MC, Ramani KC, Puthoor D. Periosteal chondrosarcoma of metacarpal bone: report of a rare entity. *Australas. Med. J.* 2012; **5**: 589–592.
36. Kumta SM, Griffith JF, Chow LTC, Leung PC. Primary juxtacortical chondrosarcoma dedifferentiating after 20 years. *Skeletal Radiol.* 1998; **27**: 569–573.
37. Matsumura T, Yamaguchi T, Hasegawa T, Kaya M, Wada T, Yamashita T. Periosteal chondrosarcoma with microscopic cortical invasion. *J. Orthop. Sci.* 2008; **13**: 379–382.
38. Nietzschman HR, Mena JC, Valle DE *et al.* Musculoskeletal case of the day. *AJR Am. J. Roentgenol.* 1998; **171**: 861–870.
39. Van Damme PHA, De Wilde PCM, Koot RAC, Bruaset I, Slotweg PJ, Ruitter DJ. Juxtacortical chondrosarcoma of the mandible: report of a unique case and review of the literature. *Int. J. Oral Maxillofac. Surg.* 2005; **34**: 94–98.
40. Weinberg J, Miller TT, Handelsman JE, Kahn LB, Godfried DH, Kenan S. Periosteal chondrosarcoma in a 9-year-old girl with osteochondromatosis. *Skeletal Radiol.* 2005; **34**: 539–542.
41. Hatano H, Ogoe A, Hotta T, Otsuka H, Takahashi HE. Periosteal chondrosarcoma invading the medullary cavity. *Skeletal Radiol.* 1997; **26**: 375–378.
42. Kenan S, Abdelwahab IF, Klein MJ, Hermann G, Lewis MM. Lesions of juxtacortical origin (surface lesions of bone). *Skeletal Radiol.* 1993; **22**: 337–357.
43. Huvos AG. Malignant surface lesions of bone. *Curr. Diagn. Pathol.* 2001; **7**: 247–250.
44. Dahlin DC, Salvador AH. Chondrosarcomas of bone of the hands and feet—a study of 30 cases. *Cancer* 1974; **34**: 755–760.
45. Bauer TW, Dorfman HD, Latham JT. Periosteal chondroma: a clinicopathologic study of 23 cases. *Am. J. Surg. Pathol.* 1982; **6**: 631–637.
46. Unni KK, Dahlin DC, Beabout JW. Periosteal osteogenic sarcoma. *Cancer* 1976; **37**: 2476–2485.
47. Hall RB, Robinson LH, Malawar MM, Dunham WK. Periosteal osteosarcoma. *Cancer* 1985; **55**: 165–171.
48. de Andrea CE, Reijnders CM, Kroon HM *et al.* Secondary peripheral chondrosarcoma evolving from osteochondroma as a result of outgrowth of cells with functional EXT. *Oncogene* 2012; **31**: 1095–1104.
49. Hameetman L, Rozeman LB, Lombaerts M *et al.* Peripheral chondrosarcoma progression is accompanied by decreased Indian Hedgehog (IHH) signalling. *J. Pathol.* 2006; **209**: 501–511.
50. van Beerendonk HM, Rozeman LB, Taminiou AHM *et al.* Molecular analysis of the INK4A/INK4A-ARF gene locus in conventional (central) chondrosarcomas and enchondromas: indication of an important gene for tumour progression. *J. Pathol.* 2004; **202**: 359–366.
51. Asp J, Inerot S, Block JA, Lindahl A. Alterations in the regulatory pathway involving p16, pRb and CDK4 in human chondrosarcoma. *J. Orthop. Res.* 2001; **19**: 149–154.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Antibodies used for immunohistochemistry.

Table S2. Primer sequences *IDH1* and *IDH2*.

Table S3. *IDH* mutation analysis: sequencing analysis and Taqman assay.

Table S4. Immunohistochemical staining of 24 periosteal chondrosarcomas.