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Original Paper

Expression of cartilage growth plate signalling molecules in chondroblastoma

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Abstract

Chondroblastoma (CB) is a rare benign tumour (<1% of all bone tumours) involving epiphyseal long bones (male:female 1.5:1). During development, and in the postnatal period, Ihh/PTHrP and FGF signalling molecules control the space and timing of chondrocyte differentiation. Considering the close relationship of CB with the growth plate (age and location), the expression of proteins involved in epiphyseal growth regulation was studied. Twelve cases of CB were retrieved. Immunohistochemistry was performed using antibodies against fibroblast growth factor-2 (FGF-2), fibroblast growth factor receptor-1 (FGFR-1), FGFR-3, bcl-2, p21, parathyroid hormone-related peptide (PTHrP), and parathyroid hormone-related peptide receptor (PTHR1). Three observers evaluated haematoxylin and eosin (H&E)-stained and immunostained slides independently. Semi-quantitative estimation of the matrix, the type of matrix, and immunostaining was performed. Cellular and matrix-rich areas were evaluated separately. Diverse amounts and types of matrix were present in different tumours, as well as within individual tumours. Signalling molecules were expressed in 50–100% of the cases. Higher levels of expression were found in cellular areas than in matrix-rich areas, especially for PTHR1, bcl-2, and FGFR-3. CB is an unusual entity affecting specific sites, showing that both Ihh/PTHrP and FGF signalling are active. Higher expression was found in cellular than in matrix-rich areas, as in the proliferating/pre-hypertrophic growth plate zone in comparison with the hypertrophic/calcifying zone. Previous studies have shown the same molecules to be expressed with a similar pattern in chondrosarcomas. The sum of the evaluated features indicates that CB is a neoplasm originating from a mesenchymal cell committed towards chondrogenesis via active growth plate signalling pathways. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: bone neoplasm; chondroblastoma; Indian Hedgehog; growth plate FGF; matrix; cartilaginous tumours

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Introduction

Chondroblastoma is an uncommon benign bone neoplasm that typically involves the epiphyses of long bones in skeletally immature individuals. It accounts for less than 1% of all bone tumours, with a definite male sex predominance (1.5:1) [1,2]. The lesion is composed of a proliferation of immature cartilage cells (chondroblasts) set within a distinctive and heterogeneous matrix. The latter can be osteoid, fibrous, or so-called 'chondroid', or even, but more focally and rarely, as clearly identifiable mature cartilage.

During development of the epiphyseal growth plate, there is proliferation, differentiation, and maturation of chondrocytes through a number of stratified physiological zones (reserve, proliferating, pre-hypertrophic/maturing, and hypertrophic) in orderly, longitudinal columns with terminal ossification and closure of the growth plate following puberty. Regulation of these physiological changes probably involves

a number of signalling pathways including a delicate paracrine feedback loop involving both Ihh/PTHrP and FGF signalling. In the embryonic growth plate, chondrocytes in transition from the proliferating to the hypertrophic zone secrete Ihh, which has to diffuse to the lateral perichondrium, where its receptor, Patched (ptc), is expressed [3]. Subsequently, PTHrP is expressed (via a still incompletely understood mechanism) in the apical perichondrium and diffuses to its receptor PTHR1, whose expression is limited to late proliferating chondrocytes. Via up-regulation of bcl-2, further chondrocyte differentiation is inhibited, resulting in suppression of Ihh secretion, which negatively influences the feedback loop. Postnatally, the Ihh/PTHrP feedback loop is restricted to the cartilage growth plate itself. In fact, Ihh diffuses to ptc, located in the hypertrophic zone, up-regulating PTHrP expression, which through binding with its receptor, also located in the hypertrophic zone, will up-regulate bcl-2 expression [3]. Thus, PTHrP fluxes influence and

control the pace of chondrocyte differentiation with subsequent regulation of longitudinal bone growth [3,4].

The FGF signalling pathway is another important factor, probably acting in concert with the pathway described above. FGF-2 is a potent mitogen for chondrocytes and stimulates extracellular matrix production. In contrast, FGFR-3, expressed in the proliferating zone of the growth plate, can be bound by FGF-18, resulting in inhibition of chondrocyte proliferation via phosphorylation of STAT-1 with subsequent nuclear expression of p21^{WAF1/CIP1}, which is an inhibitor of the cell cycle [5–7]. This probably serves to inhibit bone growth as well as chondrocyte proliferation. Ihh is repressed by FGFR-3, probably by modulation of the bone morphogenetic protein (BMP4) and ptc [8], which would promote the progression of chondrocytes to the hypertrophic zone, restricting longitudinal bone growth. While recent work on conventional central and secondary peripheral chondrosarcomas has identified putative disturbances within this signalling pathway [4], very little is known about the possible aetiological role that disruption of these feedback loops may play in chondroblastoma tumourigenesis and subsequent intra-tumoural differentiation pathways. In order to evaluate possible disturbances within these signalling pathways, we investigated the immunohistochemical expression profiles of proteins known to be involved in normal growth regulation: fibroblast growth factor-2 (FGF-2), fibroblast growth factor receptor-1 (FGFR-1), FGFR-3, bcl-2, p21, parathyroid hormone-related peptide (PTHrP), and parathyroid hormone-related peptide receptor (PTHR1). The results were compared with the expression pattern in the normal growth plate and in a previously published cohort of peripheral and central chondrosarcomas [4].

Materials and methods

Pathological material

Formalin-fixed, formic acid-decalcified (pH 2.1; 24 h), and paraffin wax-embedded archival tumour tissue was

available for routine staining and immunohistochemical analysis of 12 cases of chondroblastoma. All cases were retrieved from the surgical pathology files of the Leiden University Medical Centre, in accordance with standard laboratory practice, and examined following haematoxylin and eosin (H&E) staining to confirm the previous diagnosis. Appropriate clinical information was obtained from case notes and radiological reports. All patient material was used in a coded manner, for which code breaking and correlation with clinical data were only possible for physicians involved in treatment of the patients. The research was conducted following all local ethical guidelines.

Immunohistochemistry

Four-micrometre-thick sections were mounted on 3-aminopropylethoxysilane (APES) (Sigma, St Louis, MO, USA) and glutaraldehyde-coated slides and dried overnight at 37 °C. Details of the antibodies used for immunohistochemical analysis are listed in Table 1. Dr J Walters of Oxford Brookes University, Oxford, UK kindly provided the FGFR-1 antibody. Immunohistochemical reactions were performed as described earlier [9]. According to our experience, the decalcification method used with formic acid has no significant influence on antibody reactivity, as shown in earlier studies with these antibodies [4]. Following dewaxing, rehydration, and blocking of endogenous peroxidase, antigen retrieval was performed in all cases. For FGFR-3 and PTHrP, slides for immunohistochemical analyses were pretreated with a 0.1% trypsin solution in 0.05% calcium chloride, pH 7.4. For FGF-2, FGFR-1, PTHR1, p21, and bcl-2 (microwave antigen retrieval), sections for immunohistochemical analyses were pretreated with a 0.01 M citrate buffer, pH 6.0. Following pre-treatment, all sections were incubated overnight with the primary antibodies. Biotin-labelled rabbit anti-mouse immunoglobulins and a biotinylated HRP–streptavidin complex (DAKO, Glostrup, Denmark) were applied. Visualization was carried out in a diaminobenzidine solution (Sigma, St Louis, MO,

Table 1. Details of the antibodies used for immunohistochemical analysis

Antigen	Source	Clone	Staining	Positive control	Internal control	Dilution	Antigen retrieval
FGF-2	Transduction Laboratories	Clone 6 MC	Nuclear	Tonsil, skin	Osteoblasts, blood vessel walls, mast cells	1:125	MWO/10 min
FGFR-1	Gift*	MC	Cyt	Skin	Osteoblasts, blood vessel walls	1:2000	MWO/10 min
FGFR-3	Sigma	PC	Cyt	Skin	Striated muscle, blood vessel walls, connective tissue, osteoclasts	1:2000	Trypsin
p21	Calbiochem	MC	Nuclear	Colon	None	1:2000	MWO/10 min
PTHrP	Oncogene	PC	Cyt	Skin	None	1:25	Trypsin
PTHR1	Babco	PC	Cyt/nuclear	Skin	Blood vessel walls, osteoblasts	1:500	MWO/10 min
bcl-2	Boehringer	Clone 124	Cyt	Tonsil	Osteoblasts, lymphocytes	1:100	MWO/10 min

* See under the materials and methods section and Acknowledgements. PC = polyclonal; MC = monoclonal; Cyt = cytoplasmic staining; nuclear = nuclear staining; FGF-2 = fibroblast growth factor-2; FGFR-1 = fibroblast growth factor receptor-1; FGFR-3 = fibroblast growth factor receptor-3; PTHrP = parathyroid hormone-related peptide; PTHR1 = parathyroid hormone-related peptide receptor; MWO = microwave oven.

USA), with the addition of 0.01 M imidazole for FGFR-1. The slides were counterstained with haematoxylin. Appropriate positive control slides were prepared according to the specificity of each antibody. Internal positive controls were present in most of the histological slides, allowing evaluation of the antigenic property of tissue after decalcification. As negative controls, slides were incubated with mouse or rabbit IgG of a corresponding (iso-) type and concentration instead of primary specific antibodies.

Morphological evaluation

Slides were reviewed and scored by three of us independently (SR, JVMGB, and PCWH). Chondroblastoma diagnosis was confirmed and a search for an associated aneurysmal bone cyst (ABC) was performed. The percentage of the extension of the matrix-rich areas was estimated semi-quantitatively (0 = 0–10%; 1 = 11–25%; 2 = 26–50%, 3 = 51–75%; and 4 = 76–100%). In addition, the contribution of the different types of matrix (osteoid, chondroid, and fibrous) was estimated as a percentage. According to the literature [10], we considered as 'chondroid' deep pink matrix, less fibrillar than osteoid, and with more obvious perilacunar cartilage cell-like spaces.

Evaluation and criteria used for scoring

Only cases of chondroblastoma with an appropriate internal control for the various antibodies used were included in this study. All 12 cases retrieved from the archives met these criteria. All the tumours were scored using the summation of intensity of signal (possible range: 0 = no expression; 1 = weak expression; 2 = moderate expression; 3 = strong expression) and the number of positive cells (% tumour cells: 0 = 0%; 1 = 1–25%; 2 = 26–50%, 3 = 51–75%; 4 = 76–100%) as described previously by us [4,11,12] and others [13]. The cellular and matrix-rich areas were evaluated separately. Three different observers (SR, JVMGB, and PCWH) scored the immunostained slides separately. The three authors reviewed discrepant cases together, reaching a consensus agreement. The above-mentioned scoring system emphasizing both the staining intensity and the percentage of cells is highly reproducible in our hands and has also been used in previous studies on decalcified bone tumour specimens [4]. The mean sum score (MSS) was calculated using the SPSS 10 software package.

Particular attention was paid to giant cells, both osteoclast-like and multinucleated tumour cells. Given the heterogeneous location in the same tumours, the intensity of signal and the distribution of staining were also evaluated on a six-tiered scale: negative, weak focal, moderate focal, weak diffuse, moderate diffuse, and strong diffuse. Striking differences from the reactivity of the normal nearby osteoclasts were also recorded. The cellular region demonstrating immunopositivity was noted (nuclear, cytoplasmic,

and membranous). The sum score of intensity and percentage was calculated and the mean value reported for both cellular and matrix-rich areas. Cut-offs as applied in a previously published study [4] were used to summarize the results obtained in terms of numbers of positive and negative cases. In general, a sum score higher than 3 was considered positive, with the exception of p21 and FGFR1, for which a sum score above 0 was considered positive.

Statistical analysis

In order to evaluate correlations between matrix formation and signalling pathways and the interactions of different molecules, statistical analysis using Fisher's exact test was performed using the SPSS 10 software package.

Results

Clinical data

The male/female ratio was 3:1 and the age at presentation ranged between 11 and 30 years (mean 17 years and median 15.5 years). Four cases each involved the epiphysis of the humerus and tibia (33.3% and 33.3%), three the epiphyses of the femur (25%), and one the os ischium (8.3%). The relevant clinicopathological data are summarized in Table 2.

Morphological evaluation

All the retrieved cases fulfilled the diagnostic criteria for chondroblastoma. One case (case 10) showed the presence of both fibrous matrix and an aneurysmal bone cyst. Three tumours were composed of cellular areas only, while the remaining nine cases demonstrated both cellular and matrix-rich areas. The cellular areas were made up of polygonal cells with often-eccentric nuclei, sometimes binucleated, with coarse chromatin and eosinophilic cytoplasm. Rare spindle or stellate cells were present. Mitoses were recognized

Table 2. Clinicopathological data for the chondroblastomas used in the study

Case No	Gender	Age (years)	Bone	ABC
1	M	20	Tibia	No
2	F	30	Humerus	No
3	M	15	Humerus	No
4	M	18	Tibia	Yes
5	M	14	Tibia	No
6	F	12	Humerus	No
7	M	27	Os ischii	No
8	M	11	Femur	No
9	M	12	Femur	No
10	M	16	Femur	Yes
11	M	12	Tibia	No
12	F	17	Humerus	No

ABC = aneurysmal bone cyst; f = female; m = male.

mainly in the cellular areas (Figure 1A). The matrix-rich areas consisted of fibrous, chondroid or osteoid areas in which spindle cells, polygonal or osteoblast like cells were recognized respectively (Figure 1B). Scant matrix calcification was seen in some cases. Mature hyaline cartilage was clearly recognized in one case (case 11). The extent of the matrix-rich area (9 of 12 cases: 75%) was generally less than 50% of the surface area (66.6% of cases). Chondroid matrix alone was present in two cases (22.2%); chondroid and osteoid together in three cases (25%); osteoid and fibrous in two cases (22.2%); and chondroid, osteoid, and fibrous in one case (11.1%). Multinucleate giant cells were dispersed throughout the tumour, especially at the interface between matrix-rich and cellular areas. Giant cells showed different morphological features, mainly with regard to shape and the characteristics of the nuclei. Some showed a more oval shape and clear nuclei; others were more slender with hyperchromatic or pyknotic nuclei and more intensely eosinophilic cytoplasm, resembling apoptotic cells (Figures 1B and 1C). Size was quite homogeneous except in one case (case 6), where larger cells, with numerous nuclei, resembling osteoclasts, were present. Associated ABC was present in two cases (16.7%); haemorrhagic areas were present in most of the cases.

Immunohistochemical evaluation

The data documented below are summarized in Table 3.

FGF-2

The cellular areas were considered positive in eight cases (66.6%). The mean sum score of all cases

Table 3. Semi-quantitative scoring results specified for cellular or matrix-rich areas

Antigen	Cellular area			Matrix-rich area		
	Pos	%	MSS	Pos	%	MSS
FGF-2	8/12	66.6	3.7	6/9	66.6	4.1
FGFR-1	12/12	100	6.9	9/9	100	6
FGFR-3	11/12	91.7	5.4	7/9	77.8	4.7
p21	12/12	100	5.3	9/9	100	3.9
PTHrP	12/12	100	6.3	8/9	88.9	5.6
PTHRI	9/12	75	4.5	3/9	33.3	3.4
bcl-2	6/12	50	3.8	2/9	22.2	2.9

% = percentage of positive cases according to the set cut-off; MSS = mean sum score.

(negative and positive according to the previously mentioned cut-off) was 3.7 ± 2.6 SD. Positivity of the tumour cells was both nuclear and cytoplasmic. Matrix rich-areas (66.6%) were considered positive in six cases, the mean sum score being 4.1 ± 2.6 SD. Positivity was also both nuclear and cytoplasmic. Giant cells were generally negative (Figure 1D) except for focal weak positivity in two cases and weak diffuse positivity in one case. The staining pattern in the giant cells was cytoplasmic.

FGFR-1

The tumour cells in all cases, in both cellular and matrix-rich areas, were considered positive, with mean sum scores of 6.9 ± 0.3 SD for the cellular and 6 ± 1.1 SD for the matrix-rich areas. Positivity was both nuclear and cytoplasmic.

Giant cells were mostly positive, the intensity and the distribution being mainly diffuse and strong. Interestingly, completely negative cells were found close to very strongly staining cells (Figure 1E). The staining pattern was cytoplasmic. Nearby osteoclasts showed a more homogeneous staining pattern.

FGFR-3

Tumour cells in both the cellular areas and the matrix-rich areas demonstrated cytoplasmic positivity in 91.7% of cases, with mean sum scores of 5.4 ± 1.2 SD for the cellular (91.7%) and 4.7 ± 1.2 SD (77.8%) for the matrix-rich areas. In all cases except one, giant cells were positive, often showing weak diffuse positivity. The staining pattern was cytoplasmic (Figure 1G).

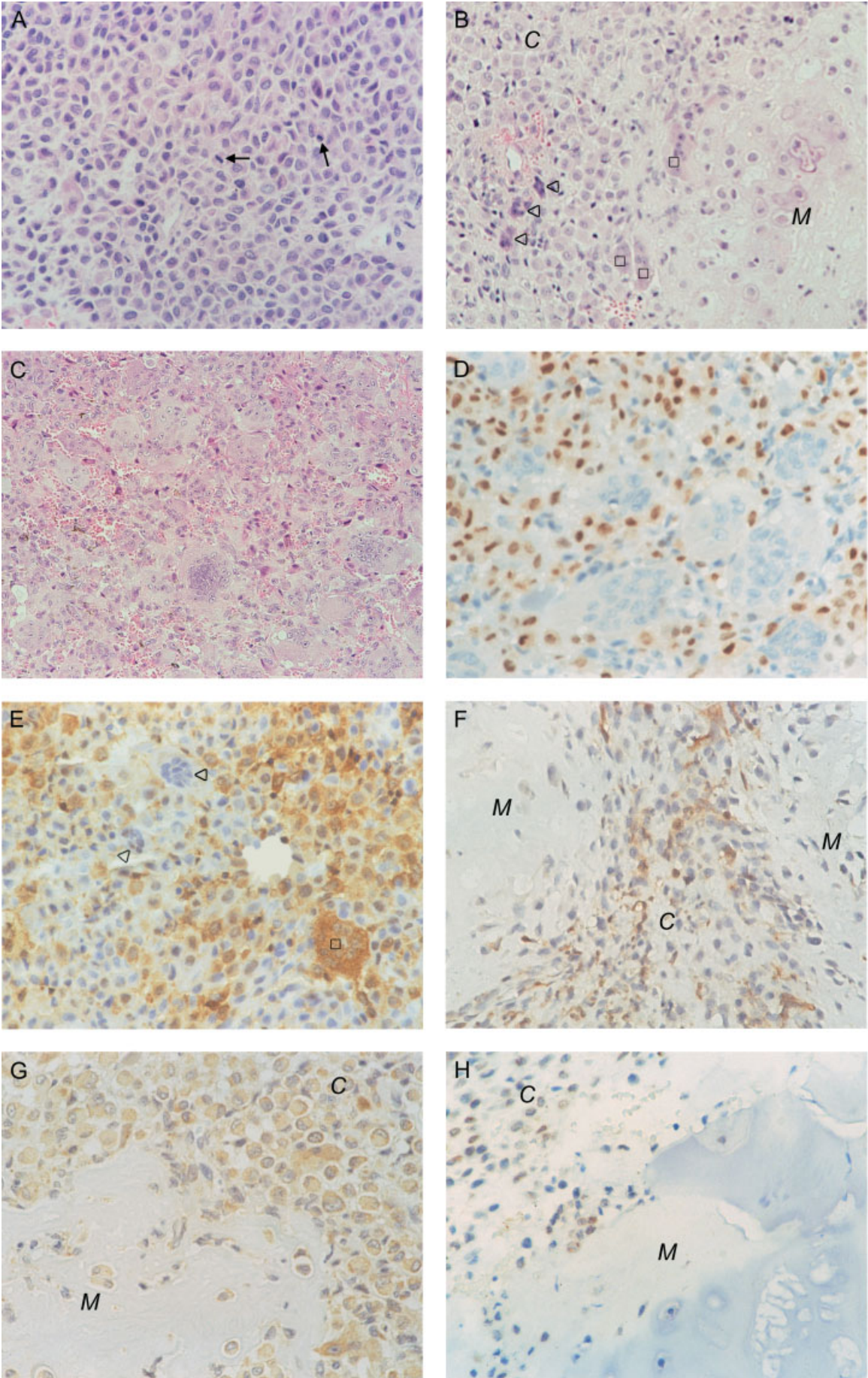
bcl-2

Positive staining of tumour cells was found in the cellular areas in six cases (50%), with a mean sum score of 3.8 ± 1.5 SD. Positivity was cytoplasmic. In the matrix rich-areas, positive staining was found in two cases (22.2%), with a mean sum score of 2.9 ± 1.4 SD. Positivity was cytoplasmic (Figure 1F). Giant cells always demonstrated cytoplasmic positivity: the intensity was weak and the distribution diffuse in six cases (50%).

p21

In all cases, tumour cells in both the cellular and the matrix-rich areas were positive, with mean sum scores of 5.2 ± 1.3 SD for the cellular and 3.9 ± 1.1

Figure 1. (A) Polygonal cells with some mitosis (arrows) are recognized in the cellular areas of chondroblastoma. (B) Matrix-rich area (M) with obvious cartilage-like cell spaces. Two different types of giant cell are recognized in the cellular areas (C): those with clear nuclei (squares) and slender cells with hyperchromatic nuclei (arrow-heads Δ). (D) Osteoclast-like giant cells with numerous clear nuclei are present. (E) FGF-2 staining is diffuse and intense in the cellular areas, whilst admixed giant cells are completely negative. (F) FGFR-1 immunostain: positive (\square) and negative (Δ) giant cells are found in the same field, while the cellular areas show diffuse positivity. (F–H) Different levels of expression between matrix-rich (M) and cellular areas (C) for bcl-2, FGFR-3 and PTHRI, respectively



SD for the matrix-rich areas. Positivity was nuclear. Giant cells were always positive, the intensity and the distribution being predominantly focal (11 cases = 91.6%). The staining pattern was nuclear. Positivity in the nearby osteoclasts was hardly found.

PTHrP

In the cellular areas, positive cytoplasmic staining was found in tumour cells in all cases, with a mean sum score of 6.3 ± 0.5 SD. In the matrix-rich areas, positive cytoplasmic staining was found in eight cases (88.9%), with a mean sum score of 5.6 ± 1.6 SD.

Giant cells were always positive, the intensity being moderate and the distribution diffuse in nine cases (75%). The staining pattern was cytoplasmic.

PTHrI

In the cellular areas, positive nuclear staining of tumour cells was found in nine cases (75%), with a mean sum score of 4.5 ± 1.5 SD. In the matrix-rich areas, positive cells were found in three cases (33.3%), with a mean sum score of 3.4 ± 1.3 SD. Positivity was nuclear and cytoplasmic (Figure 1H).

Giant cells were always positive, except in one case, and the intensity was weak and the distribution diffuse in six cases (50%). The staining pattern was nuclear.

Giant cells

Despite the fact that a diffuse or focal staining pattern was recognized in intra-tumoural giant cells, the distribution of staining was inhomogeneous in most cases, with positive cells close to completely negative ones; this was most obvious for FGFR-1. The distribution of the staining pattern was not related to the different morphological features previously described. The nearby osteoclasts showed more homogeneous staining.

Statistical analysis

Possible correlations between different amounts of matrix and types of matrix and different levels of expression of the proteins studied, and among different molecules in cellular and matrix areas, were investigated. Although the results did not reach statistical significance, a general trend can be outlined: both the percentage of positivity and the mean sum scores were higher in the cellular areas than in the matrix-rich areas, with the exception of FGF-2, where the percentage of positive cases was the same while a higher mean sum score was found in the matrix-rich areas. In particular, the difference between the percentages of positive cases (cellular versus matrix-rich areas) ranged from 0 to 42.7, being the most striking for the expression of PTHR1, bcl-2, and FGFR-3; while the difference between the mean sum scores ranged between 1.4 and 0.7, being the most striking for p21 and again PTHR1

and bcl-2. Low SD values were found (range 0.3–1.6), except for FGF-2.

Discussion

Chondroblastoma is a benign neoplasm with interesting clinical features in terms of site of occurrence and age at presentation. In the long bones, chondroblastoma mainly affects the epiphyses, while at other sites the lesion arises close to ossification centres. Chondroblastoma rarely affects bones that develop through intra-membranous ossification; hence, there is a close relationship with the presence of growth plate cartilage. Furthermore, the age of presentation is almost always before fusion of the growth plate. Males, in which this process is delayed compared with women, are more often affected.

In the context of these interactions, our analyses confirm the active role of cartilage signalling molecules in this neoplasm. For all of these proteins, there was a trend towards more extensive and intense expression in the cellular areas in comparison with the matrix-rich areas. The cellular areas are also the areas in which higher numbers of mitoses are found and hence they are more active in terms of proliferation. If we make a comparison with the chondrocytes in the growth plate, the cellular areas are most similar to the resting/proliferative zone and the matrix-rich areas to the transition/hypertrophic zone. From this perspective, the more extensive and more intense expression of the growth regulating molecules within the cellular areas reflects the prevalent expression in the proliferative zone. We compared the data from both matrix-rich and cellular areas of the chondroblastomas with our previously published data on osteochondromas, secondary peripheral chondrosarcomas, and conventional central chondrosarcomas [4]. The generally diffuse and intense expression in chondroblastomas is more similar to chondrosarcomas than to osteochondromas, in which Ihh/PTHrP and FGF signalling molecules are mostly absent [4]. This is explained by inactivation of the EXT proteins, as is the case in osteochondromas, which leads to altered expression of heparan sulphate proteoglycans (HSPG) affecting Ihh diffusion and high affinity binding of FGF to its receptors [4,14–17]. In chondrosarcomas, the re-expression of factors involved in cartilage growth and differentiation is responsible for proliferation and cell survival [4]. In chondroblastoma, the expression of growth plate signalling molecules may be due to the pre-pubertal signalling network, as well as to an implicit link with cartilage growth itself. Sex hormones are likely to be involved in this process, given the close relationship of chondroblastoma to the growth plate and its occurrence before growth plate fusion. A role for sex hormones in the pubertal growth spurt and fusion of the epiphyses is largely accepted. Hence, both osteochondroma and chondroblastoma are benign bone tumours, both of which are anatomically closely

related to the growth plate. However, while Ihh/FGF signalling is absent in the former because of the impaired expression of HSPG, caused by EXT mutations, this signalling is active in the latter. In fact, no chondroblastomas were described in patients with EXT mutations [18]. Ihh/FGF signalling is present in both chondrosarcoma and chondroblastoma; while the former is a malignant bone tumour, generally with a low proliferation rate, in which this signalling is responsible for cell survival [4], the latter is a benign bone tumour and its high proliferation rate may be the result of the activation caused by the signalling molecules. A multi-step genetic model has been postulated for osteochondroma and peripheral chondrosarcoma [19] but, to date, there is no clear molecular understanding of the tumorigenesis of chondroblastoma. For example, cytogenetic studies have shown no specific chromosomal alterations [20].

According to the WHO 2002 definition, chondroblastoma is 'a benign, cartilage-producing neoplasm usually arising in the epiphyses of skeletally immature patients' [2]. However, the nature of the matrix produced by this tumour cannot simply be viewed as cartilage, since clear mature cartilage is seldom recognizable. Moreover, the definition does not account for the extremely heterogeneous nature of the tumour. The tumours show variable features, predominantly in terms of the amount and quality of the extracellular matrix. Furthermore, different features can be recognized within the same tumour, involving the morphology of the cells, determined by the nature of the nearby matrix. So far, the evidence for the chondroid nature of this neoplasm is the morphological and ultrastructural resemblance of the main neoplastic cells to so-called chondroblasts, the scant positivity of neoplastic cells for S-100 [21,22], and the presence of proteoglycans in the extracellular matrix [23,24]. However, the presence of extracellular matrix closely resembling osteoid, the immunohistochemical positivity for collagen I, and the non-specific nature of S-100 immunoreactivity argue against a true chondroid nature. Two different studies have approached the problem of chondroid differentiation using the extracellular matrix expression profile [23,25]. Collagen II expression is considered to be the most specific marker for chondrocytes with a mature phenotype. Both groups looked for immunohistochemical positivity, achieving different results: Edel *et al* found positive expression [25], while Aigner *et al* demonstrated absence of collagen II [23], as confirmed at the mRNA expression level using *in situ* hybridization for isoform collagen II alpha 1A, the splice variant present in the early precursors of chondrocytes. From our results, chondroblastoma is a matrix-forming tumour composed of mesenchymal cells recapitulating normal chondrogenesis, at least in terms of growth plate signalling molecule expression. In view of these features, we favour the hypothesis that this neoplasm is cartilaginous in type. The production of osteoid and the positivity for collagen I could be the result of a process of transdifferentiation of chondrocytes towards

osteoblasts, as has been demonstrated in the chicken embryo [26], and is postulated to be the case for some neoplastic lesions [27].

Definitive statements concerning the cartilaginous or osseous nature of this neoplasm are not possible, in part because of the static approach offered in the literature. Attention is paid to the expression of extracellular matrix proteins, providing information about the presence or absence of a particular protein, in order to characterize the nature of the nearby cells. This kind of approach does not account for the extreme plasticity of mesenchymal cells, as they are able to modify their shape, function, and pattern of expression, through processes that have been termed 'dedifferentiation and transdifferentiation', according to the microenvironment that they are set into [28,29]. Experimental models are needed to understand further the ability of neoplastic cells to undergo transdifferentiation.

We observed two different populations of giant cells. One is probably reactive and has morphological features and immunoreactivity similar to the nearby osteoclasts. This is in agreement with previous studies that reported that histochemical and immunohistochemical markers of osteoclasts were present in the giant cells of chondroblastoma [30]. Furthermore, recent publications report the presence of RANK-ligand expression in the chondroblast-like cells of chondroblastoma [31]. This molecule, known to be expressed on the membranes of pre-osteoblastic/stromal cells, binds to its receptor RANK present on cells of the osteoclastic lineage [32]; hence, its expression in chondroblastoma suggests an active role for neoplastic cells in osteoclastogenesis.

The non-homogeneous staining pattern that we found could reflect differences in the nature of the giant cells, some being reactive and others being neoplastic. Alternatively, the different level of protein expression could simply be the result of a different functional status. Noteworthy was the expression of p21, which was barely found in the nearby osteoclasts. This is partly explained by the expression of FGFR-3, which acts upstream of this cell-cycle inhibitor [5–7]. However, both PTHR1 and bcl-2 are diffusely expressed in the giant cells, the latter being involved in anti-apoptotic processes. In chondrocytes, these two molecules are interrelated by a downstream pathway leading to increasing proliferation [33]. Hence, in intra-tumoural giant cells, both anti-apoptotic and cell cycle-inhibiting proteins are expressed, possibly as a result of a delicate feedback loop.

In conclusion, chondroblastoma is characterized by different amounts of cellular and matrix-rich areas. Both proliferative activity and signalling protein expression are greater in the cellular than in the matrix-rich areas, which are analogous to the proliferating/pre-hypertrophic zone and the hypertrophic/calcifying zone of the growth plate, respectively. According to our data, both Ihh/PTHrP and FGF signalling are active in chondroblastoma. The morphological features and immunophenotype indicate

the presence of different types of intra-tumoural giant cells in the same tumour. Taken together, the features evaluated in this study indicate that chondroblastoma is a neoplasm originating from a mesenchymal cell committed towards chondrogenesis via active growth-plate signalling pathways.

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