Short Communication

Phenotypic Diversity in Chondromyxoid Fibroma Reveals Differentiation Pattern of Tumor Mimicking Fetal Cartilage Canals Development

An Immunohistochemical Study

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Chondromyxoid fibroma represents a rare benign cartilaginous tumor of young patients occurring in a subcortical metaphyseal location. The histogenesis of chondromyxoid fibroma has not yet been postulated, even though the conventional histology and recent immunohistochemical studies on phenotype of the mesenchymal cells and extracellular matrix components suggested its origin in immature cartilage. Therefore, we wished to compare the morphological pattern of immature cartilage tissue with chondromyxoid fibroma to investigate a possible developmental counterpart of chondromyxoid fibroma. Archival paraffin-embedded tissues from 4 fetal femora and 10 cases of chondromyxoid fibroma were analyzed simultaneously using histochemistry (safranin O) and established immunohistochemical antibodies (CD34, CD163, and smooth muscle actin). Vascularized cartilage canals growing into the fetal cartilage from the perichondrium displayed characteristic glomeruloid structures with central arterioles within the immature mesenchymal stroma and numerous superficial sinusoidal blood vessels accompanied by macrophage infiltration. Similarly, each case of chondromyxoid fibroma demonstrated admixture

of two characteristic components: immature fibrous tissue of vascularized stroma with accumulation of macrophages in areas of superficial sinusoidal proliferation, and variable amounts of lobulated chondroid tissue. Based on the observed substantial morphological similarity between the cartilage canals and chondromyxoid fibroma, we suggest that the chondromyxoid fibroma represents a neoplasm originating from or mimicking the fetal cartilage canals within the immature cartilage. (Am J Pathol 2010, 177:1072–1078; DOI: 10.2353/ajpath.2010.100171)

Most parts of the human skeleton develop out of initial cartilaginous anlagen, taking form via temporally and spatially well-coordinated perichondral and endochondral ossification processes. The latter is a challenging process in that the original completely avascular cartilaginous anlage becomes highly vascularized and is eventually replaced by bone and the marrow cavity. Cartilage degradation and mineralization, angiogenesis as well as cartilage resorption coupled with apoptosis of resident cells are involved in the development of cartilage canals.¹ The canals begin as invaginations of the perichon-

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drium and invade the non-calcified cartilage matrix. They bear undifferentiated mesenchymal stem cells within an extracellular matrix, blood vessels forming glomerulus-like structures,² monocytes, chondroclasts,³ and degenerating multivacuolated cells.^{4–5} Cartilage canals are considered important for both the nutrition of the growing cartilage and elimination of waste products as well as for remodeling of the cartilaginous extracellular matrix. The regression of cartilage canals appears to be age-dependent and starts at their blind endings.⁶

Chondromyxoid fibroma is a rare benign cartilaginous bone tumor comprising less than 0.5% of all bone tumors that affect patients in all age groups, although it mostly manifests in the second decade of life.⁷ Metaphyses of long bones are most frequently affected. Chondromyxoid fibroma was first reported in 1948 by Jaffe and Lichtenstein⁸ as a benign lesion that might possibly be mistaken for chondrosarcoma. Indeed, eccentric geographic bone destruction and cortical erosion on radiographs next to histological atypia may be easily interpreted as chondrosarcoma, mostly if the tissue obtained by biopsy is small and contains tumor tissues with degenerative atypia. Macroscopically, the chondromyxoid fibroma appears mostly firm and whitish to bluish-gray, in some cases more prominently translucent or myxomatous as well. Histologically, the chondromyxoid fibroma is characterized by the presence of chondroid lobules resembling mature hyaline cartilage, myxoid areas with spindle and stellate-shaped mesenchymal cells and mononuclear histiocytoid cells next to multinucleated giant cells. The histomorphologic variability between distinct tumor areas depends on the different matrix components and their ratios; that is, a high amount of collagen restricts water binding of proteoglycan and results in a compact matrix formation. Inversely, low collagen content leads to full hydration and to the characteristic myxoid appearance of the extracellular tumor matrix.⁹

Even though the morphological resemblance of chondromyxoid fibroma with immature cartilage was discussed in earlier literature,¹⁰ the precise developmental counterpart of chondromyxoid fibroma has not yet been recognized. Therefore, we performed simultaneous conventional histological, histochemical (safranin O) and immunohistochemical analyses of archival tissues using established antibodies (CD34, CD163, α -smooth muscle actin [SMA]) on freshly cut archival tissues from both the fetal femoral epiphyses containing immature hyaline cartilage and cases of chondromyxoid fibroma. We asked whether we can recognize the potential developmental counterpart of chondromyxoid fibroma within immature hyaline cartilage to contribute to searching for a possible origin of this rare bone tumor.

Materials and Methods

The retrospective analysis of patients' records was performed in compliance with the Hamburg Hospital Law, Germany (HmbKHG, Version: 17th of April, 1991; Second chapter: Patients data protection, Paragraph 12). Paraffin-embedded archival specimens from four fetal proximal femora (Supple-

mental Table S1 available at <http://ajp.amjpathol.org>) and ten cases of chondromyxoid fibroma (Supplemental Table S2 available at <http://ajp.amjpathol.org>) diagnosed in the Institute of Pathology, University Medical Center Hamburg-Eppendorf from 2005 to 2009 were studied. All archival slides were re-reviewed and one paraffin block from each case was selected for immunohistochemistry. Freshly cut sections were stained with H&E and safranin O. Other sections were deparaffinized, enzymatically pretreated and incubated with primary antibodies (CD34 class II, clone QBEnd 10, Dako M7165, dilution 1:25; α SMA, clone 1A4, Dako M0851, dilution 1:400; CD163, clone 10D6, Novocast, dilution: 1:100) overnight at 4°C. Following this incubation, sections were incubated with Envision system (DAKO, Cytomation, Glostrup, Denmark) for 30 minutes at room temperature. Staining each sample without adding anti-human primary antibody was performed as a negative control. Finally, samples were incubated with diaminobenzidine peroxidase substrate to give a brown stain, counterstained with hematoxylin and mounted with coverslips. Intensity of the staining was graded as absent, weak, moderate, and strong.

Results

The four specimens of fetal femora in this study showed macroscopically (Figure 1A) central brown areas resembling cancellous bone within diaphysis and whitish compact elastic bone endings. Several blood vessels were apparent within the chondroid differentiated tissues. On contact radiography (Figure 1B), mineralized primary ossification centers corresponded with macroscopic osseous parts. Microscopically, the fetal cartilage tissue displayed evenly distributed round chondrocytes within a homogenous hyaline cartilaginous matrix. Superficial parts revealed a higher density of chondrocytes, however, the zonal morphology of the cartilage was not recognizable with the exception of thin areas adjacent to the primary ossification center, consistent with endochondral ossification. Here, the chondrocytes displayed clear cell morphology similar to the hypertrophic chondrocytes; however, they were not arranged in columns in all specimens except that from 24th gestational week. Multiple vascularized canals grew into the cartilage from the perichondrium. A secondary ossification center within proximal femoral epiphysis was absent at this stage of hip development. The cartilage canals were tubular and contained one central blood vessel with an apparently two- to three- layered vessel wall, fibroblastic appearing stroma and numerous superficial thin blood vessels similar to sinusoids (Figure 1C). They originated in the perichondrium and invaded the epiphyseal immature cartilage. The staining intensity for safranin O seemed to grow with each higher gestational week and in the distal direction (Figure 2, A–F). On the tip of the cartilage canals, an increased number of mononuclear cells at the interface with cartilage were apparent. Multinucleated giant cells were not prominent.

Chondromyxoid fibroma appeared macroscopically (Figure 3A) as a compact elastic tumor of whitish color

Figure 1. Proximal ending of the fetal femur (case 1; gestational week 16). **A:** The proximal femur including intertrochanteric areas showed cartilaginous differentiated bone ending. Several blood vessels originating in the perichondrium (arrows) were apparent within the cartilaginous tissue. Macroscopically, the primary ossification center was seen in the distal part of the specimen. The tan-brown appearing area (arrowhead) represents a peri-chondrial soft tissue folding between the immature trochanter and femoral neck. **B:** On contact radiography of the specimen, mineralized areas were visualized within the distal portion of the specimen. Cartilaginous tissue appeared matt and homogenous. Osseous tissue was neither present in the center of the femoral head (arrow) nor in the trochanteric region (arrowhead). **C:** Microscopically, several longitudinal and horizontally sectioned cartilage canals (arrowheads) growing from the perichondrium (arrow) within the immature cartilage displayed a typical vascular glomeruloid structure with a central arteriole, mesenchymal stroma, and superficial sinusoid associated with discrete mononuclear cellular infiltration (stain: H&E, original magnification: $\times 100$).

with a moist cut surface. Numerous intratumoral blood vessels and focal pseudocystic changes were found. Compared with the articular surface cartilage, the chondromyxoid fibroma seemed to be more elastic and moist as well as somewhat transparent. Contact radiography (Figure 3B) detected a homogenous intraosseous tumor mass and cortical scalloping in the metaphysis. In our cohort, neither the periosteal reaction nor permeation of the host bone was apparent. Histologically, the tumors contained two main components: fewer vascular chondroid lobules and focal richly vascularized areas of more cellular spindle to stellate mesenchymal tissue. The latter areas seemed focally myxomatous loose. The amount of

chondroid differentiated tumoral tissue differed between the cases but it was invariably present in each case of chondromyxoid fibroma stained with H&E (Supplemental Table S2 available at <http://ajp.amjpathol.org>). However, histochemical staining with safranin O (Supplemental Table S2 available at <http://ajp.amjpathol.org>; Figure 2, G–I) revealed a negative result in two cases and only weak to moderate positivity of a chondroid matrix in the remaining cases. More prominent chondroid tumors displayed multiple hyaline cartilaginous lobules intermingled with vascularized spindle to stellate mesenchymal stroma. Frequently, multiple sinusoid blood vessels were located parallel to the surface of nonchondroid tissue accompanied with multiple histiocytes and multinucleated giant cells. In cases with less prominent chondroid lobules, the cellularity of the spindle and stellate portions was often decreased in areas with edema or myxomatous change. In two cases (case 6 and case 9) tumor cells displayed irregular hyperchromatic (Figure 3C) or multiple nuclei, a feature similar to ancient schwannoma. Interestingly, both cases displayed only a minimal portion of chondroid differentiated tumor tissue.

In conventional histology, similar vascular structures within fibroblastic stroma were found in both the cartilage canals (Figure 4A) and chondromyxoid fibroma (Figure 4B). Immunohistochemically (Supplemental Table S1 and Supplemental Table S3 available at <http://ajp.amjpathol.org>), the endothelium of both the thick-walled central and thin-walled superficial sinusoidal blood vessels were CD34-positive in both the cartilage canals (Figure 4C) and chondromyxoid fibroma (Figure 4D). The blood vessels were organized in a similar manner, even though they were less regular within chondromyxoid fibroma cases with small portions of chondroid tissue. Similarly, a central blood vessel in the cartilage canals (Figure 4E) revealed α SMA-positive cell layers, as did the sparse central arterioles within chondromyxoid fibroma. Moreover, endothelial cells of blood capillaries were also α SMA-positive in the majority of study cases. Interestingly, scattered α SMA-positive spindle and stellate stromal cells were found within the mesenchymal stroma of the cartilage canals of all four developing bone endings and also in all cases of chondromyxoid fibroma (Figure 4F). In two chondromyxoid fibroma cases, the α SMA-positive stellate stromal cell population was prominent. Using a CD163 antibody, numerous macrophages were predominantly detected in the richly vascularized superficial areas of both cartilage canals (Figure 4G) and nonchondroid areas of chondromyxoid fibroma (Figure 4H). CD163-positive macrophages were found in every case from the study cohort. Simultaneously stained immunohistochemical negative controls showed negative results (Figure 4, I and J).

Discussion

The structural characteristics of the cartilage canals have been described in detail and have proved to be similar among vertebrates.¹¹ They are considered important for the establishment of the secondary ossification centers,

Figure 2. Extracellular matrix proteoglycans within immature proximal femoral epiphysis and chondromyxoid fibroma. **A:** Cartilage canals (arrowheads) invade the immature cartilage from the perichondrium (arrow). The extracellular stroma showed virtually no positive staining for safranin O. **B:** In the growth plate region, enlarged chondrocytes with a clear cytoplasm were present within the weakly positive chondroid matrix (**A/B:** case 1; gestational week 16). **C:** The chondroid matrix of the femoral head ending showed a weak reaction within the extracellular matrix. Both the perichondrium (arrow) and cartilage canal (arrowhead) were negative. **D:** Moderately positive staining was observed within the growth plate cartilage (**C/D:** case 3; gestational week 17). **E:** Strong safranin O staining was seen within the chondroid extracellular matrix of the last specimen. The stromal tissue of the cartilage canal (arrowhead) stained weakly positive. **F:** A similarly strong reaction and initial columnar oriented enlarged chondrocytes with clear cytoplasm were apparent within the growth plate cartilage (**E/F:** case 4; gestational week 24). The safranin O staining in chondromyxoid fibroma cases was absent (**G:** case 9), only focal and revealed moderate intensity (**H:** case 10) or more abundant (**I:** case 7). Vascularized nonchondroid appearing areas (**G-I;** arrowheads) were found in each case of chondromyxoid fibroma (stain: safranin O; **A-F,** original magnification $\times 100$; **G-I:** original magnification $\times 50$).

ensuring a normal epiphyseal development and formation of the growth plate.¹ They consist of fibroblastic mesenchymal stroma with a central arteriole and superficial sinusoids associated with both mononuclear and multinucleated giant cells. The cartilage canals invade the immature hyaline cartilage from the perichondrium.

The characteristic features of chondromyxoid fibroma are the presence of lobular chondroid areas and a somewhat more cellular vascularized fibroblastic mesenchymal tissue. In recent studies, immunohistochemical analyses have shown that tumor cells in chondroid differentiated areas are positive for S-100^{9,12–13} and YKL-40.¹⁴ Similarly, Romeo et al¹⁰ reported a striking morphological resemblance between chondroid lobules in chondromyxoid fibroma and cultured articular chondrocytes. Furthermore, the authors described different levels of the expression of BCL2, p16, N-cadherin, and cyclin D1 in both tissue types¹⁰ and concluded that the observed differences may be crucial in neoplastic chondrogenesis.¹⁰ In addition, Soder et al⁹ studied the extracellular matrix components within both the chondroid and myxoid areas and observed differences in the expression of collagen I (fibroblastic spindle cell areas) as well as collagen II and aggrecan (chondroid lobules).⁹ Interestingly, the same distribution of collagen I was described by Blumer et al¹⁵ within mouse cartilage canals. In our study cohort, the content of proteoglycans within immature epiphyseal cartilage stained with safranin O increased with a higher gestational age and in the distal direction. Interestingly, the staining intensity of safranin O within chondromyxoid fibroma cases ranged from absent to

moderate; strong staining intensity was detected in none of the study cases. We noticed a substantially lower amount of histochemically proven chondroid extracellular matrix in chondromyxoid fibroma specimens than suggested from the hematoxylin-eosin stained histological slides. Furthermore, each case of chondromyxoid fibroma revealed more or less prominent non-chondroid portions with glomeruloid vascular structures and macrophage infiltration possibly representing chondroclasts. We observed focal smooth muscle actin positive stromal cells in both cartilage canals and several chondromyxoid fibroma cases, similar with other investigators.^{14,16} The biological relevance of myofibroblastic differentiation^{16–19} in both structures is currently unknown.

Six decades after its initial description⁷ the origin of chondromyxoid fibroma has still not been established. One of the underlying reasons might be that its morphological spectrum in terms of both the type of extracellular matrix and the resident stromal cells is broader than that observed in normal mature hyaline cartilage.¹⁰ To summarize, Jaffe and Lichtenstein⁸ first suggested a fibroma with chondroid metaplasia. Schajowicz²⁰ claimed a fibromyxoid chondroma and Steiner²¹ considered chondromyxoid fibroma an intermediate neoplasm in chondroblastic differentiation between chondroblastoma and enchondroma. More recently, Soder et al⁹ suggested a specific tumor entity with a chondrogenic potential but unique biochemical composition that cannot be found in chondroblastoma, osteochondroma, enchondroma, and chondrosarcoma. Similar to Romeo et al,²² we firmly be-

Figure 3. Chondromyxoid fibroma (case 10). **A:** Macroscopically, the tumor tissue massively infiltrated the distal radius and appeared chondroid and moist. Note the difference in the consistency and color between the tumor and articular surface cartilage. A dark red area representing an organizing hematoma (star) after a previous biopsy was seen laterally. **B:** Contact radiographically, a homogenous tumor with ground-glass appearance almost completely filled the distal radius. Broad irregular cortical scalloping (arrows) can be seen. Bordering sclerosis was absent in this case. Both the periosteal reaction and permeation of the cancellous bone were absent. **C:** Microscopically, between nodular areas with chondroid differentiation, the oval to irregularly structured non-chondroid tissue (arrowhead) displayed multiple sinusoids associated with histiocyte infiltration and scattered multinucleated giant cells (stain: H&E, original magnification $\times 100$).

lieve that chondromyxoid fibroma closely resembles the development of hyaline cartilage; however, based on our observations, we do not consider the two distinct components of chondromyxoid fibroma to be a part of a spectrum of chondroid differentiation. Thus, we suggest that either component might correspond to two distinct tissue types in a composite structure that resembles the early stage of cartilage canal development within the immature hyaline cartilage before ossification centers develop. It can only be speculated whether the myxomatous changes in the nonchondroid matrix of chondromyxoid fibroma are primary features or whether they appear only secondary to vascular impaction. In our cohort, the myxomatous stroma was predominantly observed in cases with a small amount of the chondroid component.

Figure 4. Comparative immunohistochemical study on the cartilage canals (left column) and chondromyxoid fibroma (right column). **A/B:** Under low magnification, both the cartilage canals and chondromyxoid fibroma displayed a similar morphological pattern with nonchondroid areas containing blood vessels (arrowheads). **C/D:** In cartilage canals and chondromyxoid fibroma, both the superficial sinusoids and central blood vessel endothelium were positive for CD 34 (arrowheads). **E/F:** The central blood vessel wall contained few α SMA-positive cell layers in both the cartilage canals and chondromyxoid fibroma. The endothelial cells of capillaries (black arrows) also revealed a positive α SMA reaction. Similarly, scattered α SMA-positive stromal myofibroblastic cells (red arrows) were found in both lesions (**G/H**). CD163-positive macrophages (arrowheads) were characteristically seen more frequently at the tip of the cartilage canals and, both perivascular and superficially, in a manner similar to that found in chondromyxoid fibroma. **I/J:** Similar non-chondroid areas (arrowheads) displaying a glomeruloid vessel structure and mononuclear infiltration were also apparent in slides with immunohistochemical negative controls (**A/B:** H&E; **C-J:** immunohistochemical reactions; **A-J:** original magnification: $\times 100$).

It should be noted that, regarding the possible morphological relationship between chondromyxoid fibroma and the ossification center structures, several of our observations seem to be contrary to this suggestion. First, the cartilage canals within immature bone endings do not originate from the primary ossification centers, but invade the developing epiphysis directly from the perichondrium.^{1-3,5-6,11,15} At this stage of bone development, the extracellular matrix of immature cartilage tissue contains none or only a small amount of proteoglycans,

similar to chondromyxoid fibroma. The cartilage tissue from growth plate cartilage stained more intensely in our cases and, therefore, seemed more mature than the remaining chondroid extracellular matrix within the proximal femur. Furthermore, the secondary ossification centers within the femoral head occur substantially later in the postnatal development of the hip.^{23–24} Moreover, the chondrocytes in chondromyxoid fibroma neither form columnar structures nor display voluminous clear cytoplasm characteristic for the hypertrophic chondrocytes^{25–27} occurring typically within the growth plate cartilage. Indeed, we are not aware of any report on chondromyxoid fibroma with columnar organized or hypertrophic appearing chondrocyte proliferations. Therefore, we believe that our findings are fully in line with the recent observations of Soder and colleagues⁹ who could have not confirmed the suggested histogenetic concept of the chondromyxoid fibroma in growth plate remnant chondrocytes, chondroma, or chondroblasts.

Clearly, under low magnification and using a histochemical staining method for chondroid extracellular matrix proteoglycans, the architectural resemblance of the cartilage canals within immature cartilage tissue with chondromyxoid fibroma was substantial. It seemed that the more chondroid areas were present within chondromyxoid fibroma; the clearer was its resemblance with the cartilage canals within the immature cartilage. Histopathological analysis under higher magnification and immunohistochemistry with a simple panel of non-osseous and non-cartilaginous markers further supported our suggestion of possible histogenetic relationship between the cartilage canals and chondromyxoid fibroma.

We recognize several important limitations to this study. The main limitation is the limited number of analyzed cases, especially the immature cartilage tissues. Moreover, it can be suggested that similar to the developmental changes of the cartilage tissue, the extracellular matrix, blood vessels and cellular elements of the cartilage canals each undergo phenotypic changes during their life span from the initial perichondrial budding invading the immature cartilage to their later complete regression. Currently, little is known about the sequences and regression process of the cartilage canals. Therefore, we cannot conclude the precise developmental stage of the fetal immature cartilage that would possibly best correspond to chondromyxoid fibroma. More detailed analysis of these developmental processes was, however, beyond the scope of this study. Nevertheless, we strongly believe that the chondromyxoid fibroma does not simply represent a patternless or chance admixture of distinct differentiation steps of the immature hyaline cartilage but rather mimics the early developmental stages of the immature hyaline cartilage that contains vascularized cartilage canals.

Conclusion

In summary, our study demonstrated substantial similarities between the cartilage canals within the immature hyaline cartilage and chondromyxoid fibroma using a descriptive approach to the morphology of both le-

sions. Based on our results, we suggest that chondromyxoid fibroma represents a benign bone tumor originating from or mimicking cartilage canals within the immature hyaline cartilage.

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References

- Blumer MJ, Longato S, Fritsch H: Structure, formation and role of cartilage canals in the developing bone. *Ann Anat* 2008, 190: 305–315
- Wilsman NJ, Van Sickle DC: Cartilage canals, their morphology and distribution. *Anat Rec* 1972, 173:79–93
- Blumer MJ, Longato S, Richter E, Perez MT, Konakci KZ, Fritsch H: The role of cartilage canals in endochondral and perichondral bone formation: are there similarities between these two processes? *J Anat* 2005, 206:359–372
- Chappard D, Alexandre C, Riffat G: Uncalcified cartilage resorption in human fetal cartilage canals. *Tissue Cell* 1986, 18:701–707
- Rodriguez JI, Delgado E, Paniagua R: Multivacuolated cells in human cartilage canals. *Acta Anat (Basel)* 1985, 124:54–57
- Ytrehus B, Carlson CS, Lundeheim N, Mathisen L, Reinholt FP, Teige J, Ekman S: Vascularisation and osteochondrosis of the epiphyseal growth cartilage of the distal femur in pigs—development with age, growth rate, weight and joint shape. *Bone* 2004, 34:454–465
- Wu CT, Inwards CY, O’Laughlin S, Rock MG, Beabout JW, Unni KK: Chondromyxoid fibroma of bone: a clinicopathologic review of 278 cases. *Hum Pathol* 1998, 29:438–446
- Jaffe HL, Lichtenstein L: Chondromyxoid fibroma of bone; a distinctive benign tumor likely to be mistaken especially for chondrosarcoma. *Arch Pathol (Chic)* 1948, 45:541–551
- Soder S, Inwards C, Muller S, Kirchner T, Aigner T: Cell biology and matrix biochemistry of chondromyxoid fibroma. *Am J Clin Pathol* 2001, 116:271–277
- Romeo S, Bovee JV, Grogan SP, Taminiau AH, Eilers PH, Cleton-Jansen AM, Mainil-Varlet P, Hogendoorn PC: Chondromyxoid fibroma resembles in vitro chondrogenesis, but differs in expression of signalling molecules. *J Pathol* 2005, 206:135–142
- Shapiro F: Epiphyseal and physeal cartilage vascularization: a light microscopic and tritiated thymidine autoradiographic study of cartilage canals in newborn and young postnatal rabbit bone. *Anat Rec* 1998, 252:140–148
- Bleiweiss IJ, Klein MJ: Chondromyxoid fibroma: report of six cases with immunohistochemical studies. *Mod Pathol* 1990, 3:664–666
- Ushigome S, Takakuwa T, Shinagawa T, Takagi M, Kishimoto H, Mori N: Ultrastructure of cartilaginous tumors and S-100 protein in the tumors with reference to the histogenesis of chondroblastoma, chondromyxoid fibroma and mesenchymal chondrosarcoma. *Acta Pathol Jpn* 1984, 34:1285–1300
- Daugaard S, Christensen LH, Hogdall E: Markers aiding the diagnosis of chondroid tumors: an immunohistochemical study including osteonectin, bcl-2, cox-2, actin, calponin, D2-40 (podoplanin), mdm-2, CD117 (c-kit), and YKL-40. *APMIS* 2009, 117:518–525
- Blumer MJ, Longato S, Schwarzer C, Fritsch H: Bone development in the femoral epiphysis of mice: the role of cartilage canals and the fate of resting chondrocytes. *Dev Dyn* 2007, 236:2077–2088
- Romeo S, Eyden B, Prins FA, Briare-de Bruijn IH, Taminiau AH, Hogendoorn PC: TGF-beta1 drives partial myofibroblastic differentiation in chondromyxoid fibroma of bone. *J Pathol* 2006, 208:26–34
- Nielsen GP, Keel SB, Dickersin GR, Selig MK, Bhan AK, Rosenberg

- AE: Chondromyxoid fibroma: a tumor showing myofibroblastic, myo-chondroblastic, and chondrocytic differentiation. *Mod Pathol* 1999, 12:514–517
18. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G: The myofibroblast: one function, multiple origins. *Am J Pathol* 2007, 170:1807–1816
19. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G: Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. *Am J Pathol* 2001, 159:1009–1020
20. Schajowicz F, Gallardo H: Chondromyxoid fibroma (fibromyxoid chondroma) of bone. A clinico-pathological study of thirty-two cases. *J Bone Joint Surg Br* 1971, 53:198–216
21. Steiner GC: Ultrastructure of benign cartilaginous tumors of intraosseous origin. *Hum Pathol* 1979, 10:71–86
22. Romeo S, Hogendoorn PC, Dei Tos AP: Benign cartilaginous tumors of bone: from morphology to somatic and germ-line genetics. *Adv Anat Pathol* 2009, 16:307–315
23. Harcke HT, Lee MS, Sinning L, Clarke NM, Borns PF, MacEwen GD: Ossification center of the infant hip: sonographic and radiographic correlation. *AJR Am J Roentgenol* 1986, 147:317–321
24. Paranjape M, Cziger A, Katz K: Ossification of femoral head: normal sonographic standards. *J Pediatr Orthop* 2002, 22: 217–218
25. Wei L, Kanbe K, Lee M, Wei X, Pei M, Sun X, Terek R, Chen Q: Stimulation of chondrocyte hypertrophy by chemokine stromal cell-derived factor 1 in the chondro-osseous junction during endochondral bone formation. *Dev Biol* 2010, 341:236–245
26. Belluoccio D, Etich J, Rosenbaum S, Frie C, Grskovic I, Stermann J, Ehlen H, Vogel S, Zaucke F, von der Mark K, Bateman JF, Brachvogel B: Sorting of growth plate chondrocytes allows the isolation and characterisation of cells of a defined differentiation status. *J Bone Miner Res* 2010, 25:1267–1281
27. Gerstenfeld LC, Shapiro FD: Expression of bone-specific genes by hypertrophic chondrocytes: implication of the complex functions of the hypertrophic chondrocyte during endochondral bone development. *J Cell Biochem* 1996, 62:1–9