Aberrant Heparan Sulfate Proteoglycan Localization, Despite Normal Exostosin, in Central Chondrosarcoma

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The tumor suppressor genes EXT1 and EXT2 are involved in the formation of multiple osteochondromas, which can progress to become secondary peripheral chondrosarcomas. The most common chondrosarcoma subtype is primary central chondrosarcoma, which occurs in the medullar cavity of bone. The EXT1/EXT2 protein complex is involved in heparan sulfate proteoglycan (HSPG) biosynthesis, which is important for signal transduction of Indian hedgehog (IHH), WNT, and transforming growth factor (TGF)-β. The role of EXT and its downstream targets in central chondrosarcomas is currently unknown. EXT1 and EXT2 were therefore evaluated in central chondrosarcomas at both the DNA and mRNA levels. Immunohistochemistry was used to assess HSPG (CD44v3 and SDC2), WNT (β-catennin), and TGF-β (PAI-1 and phosphorylated Smad2) signaling, whereas IHH signaling was studied both by quantitative polymerase chain reaction and in vitro. mRNA levels of both EXT1 and EXT2 were normal in central chondrosarcomas; genomic alterations were absent in these regions and in 30 other HSPG-related genes. Although HSPGs were aberrantly located (CD44v3 in the Golgi and SDC2 in cytoplasm and nucleus), this was not caused by mutation. WNT signaling negatively correlated with increasing histological grade, whereas TGF-β positively correlated with increasing histological grade. IHH signaling was active, and inhibition decreased cell viability in one of six cell lines. Our data suggest that, despite normal EXT in central chondrosarcomas, HSPGs and HSPG-dependent signaling are affected in both central and peripheral chondrosarcomas. (Am J Pathol 2009, 174:979–988; DOI: 10.2353/ajpath.2009.080623)

The tumor suppressor genes EXT1 and EXT2 are known for their involvement in peripheral cartilaginous tumors. They cause the hereditary syndrome multiple osteochondromas (OMIM no. 133700), previously known as hereditary multiple exostoses.1–3 In multiple osteochondromas patients, benign cartilage capped bony outgrowths (osteochondromas) develop at the surface of bones formed by endochondral ossification. Histologically, they bear a strong resemblance to the normal growth plate. One to five percent of osteochondromas progress to (secondary) peripheral chondrosarcoma.

Although secondary peripheral chondrosarcoma is a rare chondrosarcoma subtype (less than 15% in tertiary referral centers), primary conventional central chondrosarcoma arising in the medullar cavity of bone is far more common (>80% of conventional chondrosarcoma).4,5 Enchondromas are benign cartilage tumors occurring in the center of the bone.6 Enchondromas occur mostly as solitary lesions, although they may occur as multiple lesions in the context of nonhereditary enchondromatosis (Ollier disease) (OMIM no. 166000).6

The protein products of EXT1 and EXT2, Exostosin1 and Exostosin2, are type II transmembrane glycoproteins that form a hetero-oligomeric complex, located in the Golgi membrane. This complex is responsible for elongation of heparan sulfate side chains that are linked to the
proteoglycan protein cores, consequently forming heparan sulfate proteoglycans (HSPGs). HSPGs are involved in sequestering growth factors, anchorage to the extracellular matrix, and several growth signaling pathways. Various HSPGs have been described, eg, syndecan (SDC), perlecan, and glypican families and CD44 isoforms containing HS-bearing variable exon 3 (v3). In Drosophila melanogaster, HSPGs were shown to be essential for gradient formation of the morphogens hedgehog, decapentaplegic, and wingless. The human homologues for these morphogens are Indian and Sonic hedgehog, transforming growth factor (TGF)-β, BMP, and WNT, respectively. Indian hedgehog (IHH) orchestrates chondrocyte proliferation and differentiation in the human growth plate. IHH signals to its receptor patched (PTCH), which subsequently releases its inhibition on intracellular smoothened (SMO), resulting in the translocation of GLI transcription factors to the nucleus. Here, PTHLH is transcribed together with PTCH and GLI, guaranteeing the preservation of this signaling cascade. PTHLH signaling inhibits chondrocyte differentiation and consequently controls longitudinal growth.

Although it is evident that inactivation of the EXT genes is the driving force for the development of benign peripheral cartilaginous tumors, in the far more common central chondrosarcomas the role of EXT and its downstream targets has not been systematically studied so far. We therefore investigated EXT and its downstream targets in central chondrosarcoma. Results are compared with its rare peripheral counterpart for which EXT involvement is quite well characterized. We studied EXT at the DNA and mRNA level and investigated the expression of HSPGs using immunohistochemistry. Activity of IHH, TGF-β, and WNT signaling, which require HSPGs for proper signaling, was also studied in a large series of central chondrosarcomas including six chondrosarcoma cell cultures.

Materials and Methods

Patient Material

Enchondromas and conventional central chondrosarcomas were selected based on accepted clinicopathological and radiological criteria. Peripheral, juxtacortical, mesenchymal, dedifferentiated, and clear-cell chondrosarcomas were excluded. In total, specimens of 110 patients were used for this study, including both formalin-fixed, paraffin-embedded (n = 95) and fresh frozen (n = 34) tissue. Both paraffin and frozen series included enchondromatosis (Ollier disease)-related tumors. Details are outlined in Table 1. Histological grading was performed according to Evans and colleagues. All specimens were handled according to the ethical guidelines as described in the Code for Proper Secondary Use of Human Tissue in The Netherlands of the Dutch Federation of Medical Scientific Societies.

Quantitative Real-Time Reverse Transcriptase PCR (q-RT-PCR)

q-RT-PCR was used to study the expression level of EXT1 and EXT2 and the activity of IHH signaling. Of 34 cases fresh frozen tumor tissue was available for RNA isolation, performed as described previously. Four growth plate samples, acquired from resections or biopsies for orthopedic clinical conditions not related to cartilaginous tumors, were used as controls. Results were compared with those of 28 peripheral tumors described previously. Primers for EXT1, EXT2, PTCH, GLI1, and GLI2 were described previously. Four control genes (CYP1A, CYPA, CPSF6, SRPR, and HPRT) were selected to normalize the expression data, because of their invariable expression in chondrosarcoma. Data of the cell cultures were normalized against HPRT and GAPDH. As a reference for normalization and statistical analysis a calibration curve of a mixture consisting of 15 highly diverse cell lines was included. Normalization was performed using Genorm.

Genomic Analysis

Mutation Analysis of EXT1/2 and SDC2

Two tumors with decreased EXT1 expression (L803 and L1689) were subjected to direct sequencing of the coding sequences of EXT1 and EXT2 and multiplex ligation-dependent probe amplification assay designed for EXT1 and EXT2 as described. DNA was isolated from fresh-frozen tumor tissue. DNA isolated from another set of seven tumors (four with nuclear and three with cytoplasmic SDC2 expression at immunohistochemistry) and

<table>
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<tr>
<th>Table 1. Clinicopathological Data of the 110 Patients</th>
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<tr>
<td>Total number of tumors</td>
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<tr>
<td>Grade I</td>
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<tr>
<td>Grade II</td>
</tr>
<tr>
<td>Grade III</td>
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<tr>
<td>Median age at diagnosis years (range)</td>
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<tr>
<td>Median follow-up months (range)</td>
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FFPE, formalin-fixed, paraffin-embedded.

*All fresh-frozen enchondromas were located in the phalanx.
their corresponding normal lymphocytes were screened for mutations by direct sequencing analysis of the five exons of the coding region of the SDC2 gene. Primer sequences are listed in Table 2.

Array Comparative Genomic Hybridization

L803 and L1689 were also analyzed by array comparative genomic hybridization using a high-resolution 8q array containing a tiling bacterial artificial chromosome clone set as described. In addition, the seven tumors that were subjected to mutational screening for SDC2 were hybridized to a custom-made HD-Agilent (Agilent Technologies, Amstelveen, The Netherlands) oligonucleotide array containing 4 × 44,000 immobilized 60-mer oligonucleotides. Genomic intervals containing 35 genomic regions of 37 genes selected on their function in cartilage biosynthesis (Supplementary Table S1 at http://ajp.amjpathol.org) were subjected to Agilent’s eArray web tool (Agilent Technologies) to generate oligonucleotide (60 mer) microarray probe sequences. For each gene of interest probes were selected both for exonic and intronic regions, additional probes sequences were selected 5000 bps before and after targeted genes to include potential control elements, such as promoter regions. A total 37,040 oligonucleotide probes were selected in genomic regions, additional probes up to 44,000 were added as internal control selected as a default from the array design options. Slides were printed and produced by Agilent Technologies using standard company protocols available as custom service. Array probes, layout, and access to purchase this microarray from Agilent array can be obtained from the responsible author (K.S.). Test and reference samples were labeled, hybridized, washed, scanned, and analyzed following standard protocols provided by Agilent Technologies.

Immunohistochemistry

For 95 tumors formalin-fixed, paraffin-embedded material was available (Table 1). All were stained for PAI-1 and a subset of 19 tumors for phosphorylated Smad2 to evaluate TGF-β signaling. In addition all were stained for β-catenin, to evaluate canonical WNT signaling. HSPG expression (SDC2 and CD44v3) was evaluated in a subset of 30 tumors. Subsets were representative for the total group of patients. Details of primary antibodies are described in Table 3. PAI1, phosphorylated Smad2, and β-catenin immunohistochemistry were scored semiquantitatively as described previously by two observers (Y.M.S., J.V.M.G.) independently. Both were blinded toward clinicopathological data. In brief, scores were given for β-catenin, PAI1, and phosphorylated Smad2 intensity (1 = weak, 2 = moderate, 3 = strong) and for percentage of positive cells (1 = 0 to 24%, 2 = 25 to 49%, 3 = 50 to 74%, and 4 = 75 to 100% positive cells in the total tumor section). To avoid tumors with single positive cells being regarded as positive, cutoff levels for statistical analysis were applied corresponding to general staining pattern (sum of score β-catenin ≥2 and PAI1 ≥3). SDC2 and CD44v3 were evaluated based on presence or absence and localization of staining as described. Specificity of

Table 2. Primer Sequences for SDC2 Mutation Analysis

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<th>Sequence</th>
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Table 3. Antibodies Used for IHC, WB, and IF

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<th>Positive control</th>
<th>Staining</th>
<th>Antibody concentration</th>
<th>Antigen retrieval*</th>
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<td>CD44v3</td>
<td>Labvision</td>
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<td>Tonsil</td>
<td>Membrane, cytoplasm</td>
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<td>58K Golgi protein</td>
<td>Abcam Ltd.</td>
<td>Polyclonal</td>
<td>Tonsil</td>
<td>Perinuclear Membrane, ECM</td>
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<td>Monoclonal</td>
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<td>Nuclear</td>
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*Antigen retrieval was performed using citrate buffer at 98°C for 20 minutes.
the SDC2 antibody for membranous staining was tested using a tissue microarray containing 79 soft tissue tumors of 28 different entities, as was described previously.32

**Immunoblotting**

To confirm the nuclear localization of SDC2 as shown by immunohistochemistry two fresh central chondrosarcoma samples were digested overnight in dissociation medium containing 0.1% collagenase (Sigma, Zwijndrecht, The Netherlands) and 0.1% dispase (Life Technologies, Breda, The Netherlands), 100 IU/ml. Cell fractionation was performed using two different lysis buffers. To extract cytoplasmic proteins a buffer containing 10 mmol/L HEPES pH 7.9, 10 mmol/L KCl, 0.1 mmol/L EGTA, 0.1 mmol/L EDTA, 1 mmol/L dithiothreitol, 0.5 mmol/L phenylmethyl sulfonyl fluoride, 2 µg/ml leupeptin, 1 µg/ml pepstatin A, and 2 µg/ml aprotinin was used, followed by the addition of 10% Nonidet P-40. Subsequently, the extraction of nuclear proteins was performed by vigorously rocking the sample for 15 minutes in the presence of a buffer containing 20 mmol/L HEPES, pH 7.9, 400 mmol/L NaCl, 1 mmol/L EGTA, 1 mmol/L EDTA, 1 mmol/L dithiothreitol, 1 mmol/L phenylmethyl sulfonyl fluoride, 2 µg/ml leupeptin, 1 µg/ml pepstatin A, and 2 µg/ml aprotinin. Protein concentrations were measured using a DC Protein Assay (Bio-Rad, Hercules, CA). Ten µg of each sample were run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were electrophoretically transferred onto polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, MA). Membranes were pre-incubated with 15% skinned milk in Tris-buffered saline/Tween. After incubation with first and secondary antibodies (Table 3), the membranes were developed with enhanced chemiluminescence Western blotting detection reagent (Amersham Biosciences, Buckinghamshire, UK) and visualized by exposure to X-ray films (Hyperfilm ECL, Amersham Biosciences).

**Confocal Microscopy and Immunofluorescence**

To confirm localization of CD44v3 in the Golgi apparatus, two central chondrosarcomas (with perinuclear CD44v3 expression) were selected for fluorescent double staining using CD44v3 and the 58K Golgi protein antibodies as described previously.22

**Hedgehog Activity in Vitro**

Chondrosarcoma cell lines CH2879,33 C3842,34 OUMS27,35 and SW1353 (American Type Culture Collection, Manassas, VA), central chondrosarcoma primary cultures L784 and L869, and cyclopamine-responsive pancreatic carcinoma cell line PANC1 (American Type Culture Collection)36 were used to analyze HH signaling. Chondrosarcoma cell lines were cultured in RPMI 1640 and PANC1 in Dulbecco’s modified Eagle’s medium (both Gibco, Invitrogen Life-Technologies, Scotland, UK) supplemented with 10% heat-inactivated fetal calf serum (Gibco, Invitrogen Life-Technologies) at 37°C in a humidified incubator with 95% air and 5% CO2. Cartilaginous phenotype was confirmed by RT-PCR, showing mRNA expression of collagens I, 2B, 3, and 10; Aggrecan; and SOX9.37

For RNA analysis, 2.5 x 106 cells were seeded in a six-well plate. After 24 hours the medium was replaced with serum-starved medium (0.05% fetal calf serum) con-

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**Figure 1.**

A: EXT1 mRNA expression levels of central chondrosarcoma are comparable with growth plate levels whereas peripheral tumors (published previously16) show a threefold decrease. B: EXT2 mRNA levels are slightly higher in central and slightly lower in peripheral chondrosarcoma, compared with growth plate. Expression levels of the tumors are log2 transformed and expressed as relative to the growth plate levels. C: 8q tiling array comparative genomic hybridization pattern of central chondrosarcoma L803 is shown. Genomic alterations are absent, despite low EXT1 expression by qPCR. L1689 showed similar results as L803 (data not shown). For comparison, an osteochondroma with homozygous loss of multiple clones covering the EXT1 gene and hemizygous loss of a larger part of 8q is shown in D (figure composed of data published previously16).
taining either 10 μmol/L cyclopamine (Toronto Research Chemicals, North York, Canada) or dimethyl sulfoxide 0.1%. Cells were harvested after 24 hours and RNA was isolated using Trizol and microspin column (Qiagen, Hilden, Germany). Cell viability was assessed by WST-1 colorimetric assay (Roche Diagnostics GmbH, Penzberg, Germany), which measures mitochondrial activity. Cells were seeded into 96-well flat-bottom plates (1.5 × 10³ cells/well for SW1353 and PANC1 and 5.0 × 10³ cells for CH2879, C3842, OUMS27, L784, and L869). After 24 hours dimethyl sulfoxide, cyclopamine at 5 μmol/L and 10 μmol/L, and tomatidine (Toronto Research Chemicals), an inactive but structurally related compound,38 at 10 μmol/L, were added in the presence of 5% fetal calf serum, each condition in quadruplicate. After 3 days of treatment, the metabolic activity of the cells was measured on a Victor3 Multilabel Counter 1420-042 (Perkin Elmer, Waltham, MA) at 450 nm.

Statistical Analysis

Correlations between histological grade and immunohistochemical results were calculated by χ² test. Differences in cell viability in vitro were calculated by Student’s t-test. P values <0.05 were considered significant.

Results

Normal EXT1 and EXT2 in Central Chondrosarcoma

The expression of EXT1 and EXT2 mRNA in central cartilaginous tumors is not decreased as compared with growth plate samples, which is in contrast to the low EXT1 expression levels previously found in peripheral tumors (Figure 1, A and B).22 No correlation of EXT1 or EXT2 expression with histological grade was found (not shown). Direct mutation screening and MLPA for both EXT1 and EXT2 did not reveal any genetic aberrations (not shown). Comparative genomic hybridization on an 8q bacterial artificial chromosome tiling array of the two central chondrosarcomas with the lowest expression of EXT1 did not reveal any alterations on 8q24, the locus for EXT1. Results for L803 are shown in Figure 1, C and D, which are representative for L1689 (not shown). No other gains or losses in 8q were detected. Moreover, no losses were found in the EXT1 and EXT2 genes in the seven chondrosarcomas hybridized at the oligonucleotide Agilent array.

Aberrant Location of HSPGs SDC2 and CD44v3 in Central Chondrosarcoma

Cytoplasmic staining of CD44v3 was found in 26 of 30 (87%) central tumors (7 of 8 EC and 14 of 22 CS). The staining was dot-like suggesting localization in the Golgi apparatus (Figure 2A), which was confirmed using immunofluorescent confocal microscopy (Figure 2B). Central chondrosarcomas showed cytoplasmic staining of SDC2 in 28 of 29 (97%). Moreover, additional nuclear staining, shown in Figure 2C, was found in half of the tumors (4 of 7 EC and 11 of 23 CS). The soft tissue tumor tissue microarray did not show nuclear staining in any of the specimens (not shown). Control sections of tonsil showing membranous expression pattern of CD44v3 in the epithelial layer and normal growth plate showed SDC2
staining at the cell membrane and in the extracellular matrix surrounding hypertrophic chondrocytes (not shown). Nuclear localization of SDC2 as found by immunohistochemistry was verified in the nuclear component of the cell fractionation by immunoblotting (Figure 2D).

No Aberrations in Other Genes Important for HSPG Formation

Because we found normal EXT in central chondrosarcomas, we searched for aberrations in other regulators of HSPG formation. Mutation screening of the five coding exons of the SDC2 gene did not reveal any mutations in the seven tumors (not shown). Using the oligonucleotide tiling array covering 30 EXT, EXT-like, and other genes involved in HSPG biosynthesis we did not find any specific genomic losses or gains in these seven tumors.

Decreased WNT Signaling and Increased TGF-β Signaling in High-Grade Chondrosarcomas

Nuclear staining for β-catenin (Figure 3A) was found in 17% (5 of 30) of enchondromas, in 47% (11 of 23) of grade I, and in 29% (7 of 24) of grade II and in 11% (1 of 9) grade III chondrosarcoma (Figure 3B). The activity of canonical WNT signaling was increased in grade I chondrosarcomas compared with enchondromas. However on further increase in histological grade the activity decreased again (Pearson $\chi^2$, $P = 0.038$). Expression of PAI-1, implicating active TGF-β signaling (Figure 3C), was associated with increased histological grade (Pearson $\chi^2$, $P = 0.002$). All high-grade tumors were positive (35 of 35), whereas the enchondromas and low-grade chondrosarcoma were positive in 73% (24 of 33) and 74% (20 of 27), respectively (Figure 3D). Moreover, nuclear localization of phosphorylated Smad2 was demonstrated in all of 6 enchondromas and 13 chondrosarcomas, suggesting active TGF-β signaling (not shown).

HH Signaling in Central Chondrosarcoma in Vivo and in Vitro

By qPCR active HH signaling was shown in central chondrosarcoma, irrespective of histological grade, because PTCH, GLI1, and GLI2 were expressed at similar levels as in the growth plates (Figure 4, A–C). Cyclopamine treatment resulted in a profound decrease of GLI1 mRNA expression combined with decreased cell viability in only one cell culture (CH2879), whereas all other cultures did not respond by decreased GLI1 levels or cell viability (Figure 5A). In L869, an increase of GLI1 was observed whereas L784 showed increased cell viability. In all chondrosarcoma cell cultures PTCH levels were unchanged or even increased after cyclopamine treat-
ment (Figure 5B). The viability of the tomatidine controls showed that the decrease in viability in PANC1 and CH2879 is not a toxic side effect of the cyclopamine compound (Figure 5C).

Discussion

We present the first systematic evaluation of EXT and its downstream targets in central chondrosarcoma. We confirm that peripheral and central chondrosarcomas are clear distinct genetical entities also with respect to their EXT expression. Whereas inactivation of EXT is the driving force for the development of benign peripheral cartilage tumors,22 we demonstrate in central cartilage tumors the EXT genes to be normal both at the DNA as well as at the mRNA expression level. The absence of larger deletions in the EXT1 region is in concordance with the previously reported absence of loss of heterozygosity31 and karyotypic aberrations39 at 8q24 in central chondrosarcomas.

The EXT proteins are involved in the biosynthesis of heparan sulfate. Despite normal expression of the EXT genes in central tumors, heparan sulfate proteoglycans (CD44v3 and SDC2) unexpectedly accumulated in the cytoplasm. This is similar to what we previously described for peripheral tumors carrying EXT mutations,22 whereas in other tumors membranous expression of CD44v3 and SDC2 is described.40,41 In addition, 50% of central chondrosarcomas also demonstrated nuclear SDC2 expression, which was not observed in peripheral chondrosarcomas, nor in 79 soft tissue sarcomas. In addition to the general function of SDC2 in modulating extracellular ligands, intracellular actions are implicated for SDC2. In osteosarcoma SDC2 has been shown to induce apoptosis.42,43 We excluded mutations in the nuclear localization signal, located at the first exon of the SDC2 gene, however other mechanisms such as aberrant phosphorylation of the nuclear localization signal or...
mutations in a kinase responsible for the phosphorylation of the nuclear localization signal might explain the aberrant localization of SDC2 in central chondrosarcoma.

In addition, it is tempting to speculate that, for example, other glycosyltransferases than EXT or the sulfotransferases or epimerases that function to complete the formation of heparan sulfate proteoglycans are affected in central chondrosarcoma. By using a custom-designed oligonucleotide tiling array of 30 EXT, EXT-like, and other genes involved in HSPG biosynthesis we excluded that neither heterozygous nor homozygous losses, nor gains, are found in these genes. Nonetheless, we cannot exclude that these HSPG biosynthesis-related genes might be subjected to point mutations, or other copy number neutral alterations, ie, inversion or methylation.

In Drosophila, HSPGs are essential for gradient formation of the morphogens hedgehog, decapentaplegic, and wingless. In humans, the IHH pathway, through PTHLH signaling, is vital for chondrocyte proliferation and differentiation in the growth plate. We show hedgehog signaling to be active in central chondrosarcoma. We previously demonstrated PTHLH signaling, which is downstream of IHH, to be active in central chondrosarcomas as well. In osteochondromas, IHH signaling is active, whereas activity decreases in peripheral chondrosarcoma with increasing histological grade. Tiet and colleagues reported active IHH signaling in chondrosarcoma, but did not distinguish between peripheral and central tumors. In a previous pilot series we reported lower levels of IHH signaling in central tumors as compared with the growth plate. In the present larger series IHH signaling levels are comparable to growth plate samples. Moreover, in one of the cell lines, CH2879, an important role for hedgehog signaling in cell proliferation could be shown because inhibiting HH signaling using cyclopamine decreased cell viability. IHH signaling has been shown to be activated in many cancers such as medulloblastoma, basal cell carcinoma, small-cell lung cancer, breast cancer, and pancreatic cancer. Therefore, targeting IHH signaling seems to be promising in cancer therapy. Previously, Tiet and colleagues also showed that blocking HH signaling using either cyclopamine or triparanol, an inhibitor of 7-dehydrocholesterol reductase, reduced proliferation and tumor volume in 10 chondrosarcoma xenografts. However, we observed an effect of IHH blockade using cyclopamine only in one of six chondrosarcoma cell cultures. Surprisingly, PTCH was found to be increased in those cultures that were resistant to cyclopamine, which might be induced by other pathways. These data suggest that despite aberrant cellular HSPG distribution, IHH signaling is active in central chondrosarcoma and it is vital for cellular proliferation and therefore a putative therapeutic target in a small subset of them. Because HSPGs are thought to be important for the diffusion of HH to its receptor on target cells, it might be that in central chondrosarcoma these diffusion problems are overcome by cell autonomous (autocrine) HH signaling, as was previously also suggested for osteochondromas.

The activity of other signaling pathways dependent on HSPG was similar to the activity in peripheral chondrosarcoma. Our results suggest that active canonical WNT signaling might be important for the transition from enchondroma toward low-grade central chondrosarcoma, however it is not crucial for progression toward higher grade (Figure 6). In contrast, TGF-β signaling was shown to increase with increasing histological grade by both the presence of PAI-1 and the finding of nuclear localization of phosphorylated Smad2. This suggests that TGF-β has a role in either rearrangement of extracellular matrix and/or vessel formation, which are the main characteristics of high-grade chondrosarcoma.

In conclusion, we present a systematic investigation of EXT and its downstream targets in central chondrosarcoma. We clearly show that the EXT genes are normal in central chondrosarcoma, with nevertheless aberrant localization of HSPGs. Despite this, IHH signaling is active in central chondrosarcoma and is important for proliferation and therefore a potential therapeutic target in a small subset of central chondrosarcomas. The aberrant intracellular accumulation of HSPG in central chondrosarcoma which is similar to peripheral chondrosarcoma and the nuclear SDC2 localization that is exclusively seen in central chondrosarcoma is difficult to explain. Nevertheless, our data, summarized in Figure 6, suggest that a disturbed HSPG functioning is involved in the histogenesis of both central and peripheral chondrosarcoma, although different steps in HSPG biosynthesis seem affected.
Acknowledgments

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