A screening based approach to find new paths for targeted treatment in chondrosarcoma

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Chapter 2

Molecular drivers in chondrosarcoma

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Introduction

Chondrosarcoma is a malignant cartilage forming tumour accounting for 20% of all malignant bone tumours [1]. Only a small percentage of chondrosarcomas is found in the skull or spine (2-12%) [2]. Depending on the subtype, different molecular pathways are involved in development and progression of chondrosarcoma. In the skull or spine, chondrosarcoma needs to be distinguished from chordoma, which shows a similar presentation and anatomical location, but a different biological behaviour. The distinction can be made using brachyury immunohistochemistry, as overexpression of brachyury is the hallmark of chordoma [3]. On the other hand, chondrosarcomas represent a heterogeneous group of tumours, in which different cell signalling pathways are involved in tumorigenesis. In this chapter, the most important pathways will be discussed.

Clinicopathological classification of chondrosarcoma

Chondrosarcomas can be divided in conventional chondrosarcoma (85%), and more rare subtypes; dedifferentiated chondrosarcoma (10%), mesenchymal chondrosarcoma (2%), clear cell chondrosarcoma (2%) and periosteal chondrosarcoma (1%) [4-7]. Central conventional chondrosarcoma is the most common in the skull and spine, followed by mesenchymal chondrosarcoma [8].

Conventional chondrosarcoma

Conventional chondrosarcoma can be further subdivided in central (>85%) and peripheral chondrosarcoma (~15%) based on its location in the bone. Central chondrosarcomas develop in the bone either directly or from a previously developed benign enchondroma [4] (see figure 1). Enchondromas can develop as a solitary lesion or as multiple enchondromas in Ollier disease or Maffuci syndrome [9]. Ollier disease and Maffuci syndrome are both rare non-hereditary disorders caused by somatic mosaic mutations in *IDH1* or *IDH2*. In solitary enchondromas ~1% undergoes malignant transformation towards a conventional central chondrosarcoma. Enchondromas from patients with multiple enchondromas have a much higher transformation rate of ~50% [10].

Peripheral chondrosarcomas develop at the surface of the bone from a pre-existing benign osteochondroma. They develop from bones that are formed by endochondral ossification; consequently they are extremely rare findings in the skull. Central and peripheral chondrosarcomas show similar histological features and are graded using a three tiered scale. Atypical cartilaginous tumours (previously chondrosarcoma grade I) show low cellularity, do not metastasize, but behave locally aggressive and local recurrences can develop. Grade II and III chondrosarcomas are high grade tumours with an increased cellularity and mitoses. These tumours show a higher metastatic rate and poor patient survival. Patients with atypical cartilage tumours show a 10 years overall survival rate of 83%, while patients with grade II chondrosarcomas show a 10 years survival rate of 64% and patients with a grade III chondrosarcoma show the poorest 10 years survival rate of 29% [4, 11] So far, histological grade is the most important predictor of prognosis. Upon recurrence of low grade tumours, progression towards a higher histological grade can occur in ~13% of the cases [12, 13], resulting in a poorer prognosis. Chondrosarcomas are resistant to conventional chemo- and radio therapy, leaving surgical removal as the only treatment option [14]. This has major implications for patients with inoperable disease to whom no treatment options can be offered.

Rare chondrosarcoma subtypes

Dedifferentiated chondrosarcoma comprises ~10% of all chondrosarcomas [5]. It is a high grade chondrosarcoma variant that is characterized by a mixed histological appearance of dedifferentiated cells and a cartilaginous component. Patients with dedifferentiated chondrosarcoma show a 5 years overall survival between 7 and 24% [5, 15]. Like conventional chondrosarcoma, dedifferentiated chondrosarcoma is also resistant to conventional chemo- and radiotherapy. Mesenchymal chondrosarcoma is a rare high grade chondrosarcoma consisting of differentiated cartilage mixed with undifferentiated small round cells [7]. Ten years survival rates are reported between 27 and 67% [16, 17]. The small round cell component of mesenchymal chondrosarcoma may respond better to chemotherapy compared to other chondrosarcoma subtypes [17, 18]. Clear cell chondrosarcoma is a rare low grade chondrosarcoma mainly found in the epiphysis of the humoral or femoral head [11]. Histologically, clear cell chondrosarcoma shows malignant cells with clear empty cytoplasm surrounded by hyaline cartilaginous matrix [6]. Surgery is the only treatment option for patients with clear cell chondrosarcoma. The prognosis

is relatively good with a mortality rate of 15% [6]. Periosteal chondrosarcoma is the rarest chondrosarcoma subtype accounting for less than 1% of the cases and originates from the periosteum [19, 20].

Molecular drivers in chondrosarcoma

In this chapter the molecular pathways and mutations in chondrosarcoma are discussed for the different chondrosarcoma subtypes, however most research has been focussing on central conventional chondrosarcoma. Mutations in *IDH1* and *IDH2* have been found in central as well as dedifferentiated and periosteal chondrosarcoma. *COL2A1* mutations and alterations in Indian hedgehog genes are seen in a small percentage of central chondrosarcoma. Deregulation of the hedgehog pathway has been found in a large part of chondrosarcomas indicating that it is important for the development of these tumours. Peripheral chondrosarcomas are characterized by mutations in *EXT1* or *EXT2*. Both central and peripheral chondrosarcomas show alterations in p53 and pRb pathways when progressing from low to high grade.

Central, dedifferentiated and periosteal chondrosarcoma

IDH1 and *IDH2* mutations

Mutations in *isocitrate dehydrogenase 1* and *2* (*IDH1* and *IDH2*) have been first identified in glioblastoma multiforme [21] and later also in 87% of enchondromas, 38-70% of primary central chondrosarcomas, 54% of dedifferentiated chondrosarcomas and 15% of periosteal chondrosarcomas [9, 19, 22, 23]. The occurrence of *IDH1* and *IDH2* mutations in enchondroma indicates that this is an early genetic event in the development of central chondrosarcoma (see figure 1). Also in intracranial chondrosarcomas, but not chordomas, *IDH1* and *IDH2* mutations have been reported, making it a good diagnostic marker in the differential diagnosis between the two different entities [24, 25].

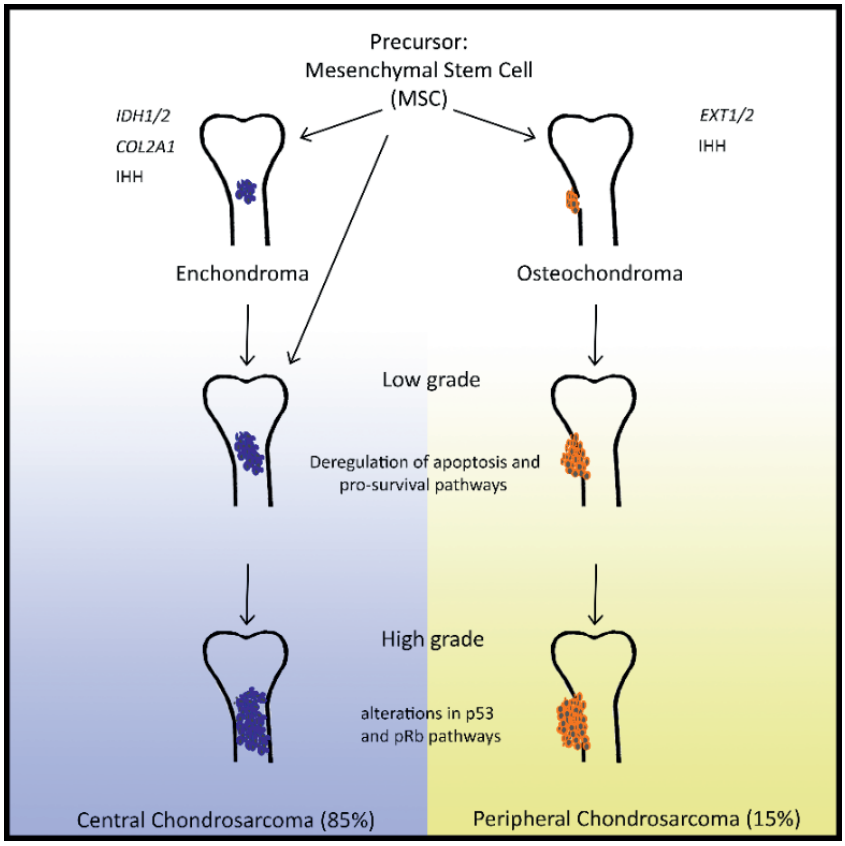

Figure 1. Progression model of conventional chondrosarcoma
Enchondromas, located in the medulla of the bone, can develop into central chondrosarcoma, while osteochondromas, located at the surface of the bone, can progress into peripheral chondrosarcoma. Early driver gene alterations include mutations in *IDH1* or *-2* in enchondroma and *EXT1* or *-2* in osteochondromas. Upon progression towards chondrosarcoma, similar molecular defects in apoptosis and survival pathways are observed in central and peripheral subtypes. Deregulation of p53 and pRb pathways is observed in the majority of high grade chondrosarcomas.

Cellular consequences of an *IDH1* or *IDH2* mutation

IDH1 and IDH2 are enzymes that convert isocitrate to alpha ketoglutarate (αKG) in the Kreb's cycle. IDH1 is located in the cytoplasm and IDH2 in the mitochondria and they both convert isocitrate to αKG while meanwhile NADPH is produced from NADH+ [26]. Mutations in *IDH1* and *IDH2* are heterozygous and affect only a single amino acid. In chondrosarcoma, the *IDH1* mutation is always located on the R132 position in exon 4, and the *IDH2* mutation on R172, which are the active sites of the enzymes. *IDH1* and

IDH2 mutations are mutually exclusive. When IDH1 or IDH2 are mutated, isocitrate cannot be converted to αKG anymore, because affinity for isocitrate is reduced. However, affinity for αKG and NADPH is increased, which leads to the conversion of αKG to the oncometabolite D2-hydroxyglutarate (D2HG) which consumes instead of produces NADPH. Also the enzyme activity of the wild type IDH is inhibited by the mutant [27, 28].

D2HG production has major consequences for enzymes that depend on αKG as a co-substrate (2-OG-dependent dioxygenases). D2HG and αKG are structurally the same, except that the C2 carbonyl group of αKG is replaced by a hydroxyl group in D2HG. Because of the similarity of D2HG to αKG, D2HG acts as a competitive inhibitor by binding to the αKG binding pocket in the active site of the enzymes that depend on αKG [27, 29].

One group of enzymes affected by increased D2HG levels are the ten-eleven translocation (TET) enzymes (Tet 1/2/3). Tet enzymes are demethylating enzymes that convert 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), which is the first step in demethylation of cytosines [30-32]. Several studies have been conducted investigating the role of Tet enzymes and 5-hmC, showing that they play an important role in stem cell development and differentiation. Simultaneous knock down of all three Tet proteins in mouse ES cells resulted in low levels of 5-hmC, impaired differentiation, promoter hypermethylation and deregulated expression of genes involved in embryonic development [33].

JMJC domain containing histone lysine demethylases (JHKDMs) are also inhibited by D2HG. These enzymes remove methyl groups from the lysine residues of histone proteins, which will affect the histone code leading to alterations in chromatin structure and transcription of genes [29, 34, 35].

Another group of αKG dependent enzymes influenced by D2HG are collagen and HIF prolyl-4hydroxylases (PHDs) [29, 36]. Collagen PHDs are important for collagen folding and maturation. When these enzymes are inhibited collagen cannot be properly folded and accumulates in the endoplasmic reticulum (ER). Hydroxylated collagen is part of the extracellular matrix (ECM), which can influence proliferation, differentiation and invasion [37].

HIF PHDs are oxygen sensing enzymes that hydroxylate HIF1α and HIF2α under normoxic conditions, marking them for recognition by the Von Hippel Lindau (VHL) protein, which targets them for proteosomal degradation. Under hypoxic conditions there is no hydroxylation and HIF1α and HIF2α can dimerize with HIF1β to form complete transcription factors targeting

genes that function to increase oxygen delivery or enhance angiogenesis [38]. Since HIF PHDs need αKG to hydroxylate HIF1α and HIF2α, the hypothesis is that *IDH1* or *IDH2* mutant tumours show a higher frequency of HIF1α and HIF2α activation, because they will not be recognized by VHL and targeted for degradation. However conflicting results have been shown about the role of HIF PHDs in IDH mutant tumours, especially in glioblastomas, reporting both up and down regulation of HIF1α and HIF2α in *IDH1* or *IDH2* mutant tumour cells compared to wild type tumour cells [29, 36, 38]. This indicates that a more complex mechanism is involved in the role of HIF PHDs in *IDH1* or *IDH2* mutant tumours [39].

Mutations in *IDH1* or *IDH2* are expected to alter the metabolism of cells, because IDH1 or IDH2 are involved in the Krebs cycle converting isocitrate to αKG or the other way around. Mutations in IDH1 or IDH2 will inhibit this process and thereby cause differences in metabolic processes that depend on the Krebs cycle, such as glucose and glutamine metabolism as well as fatty acid production [40].

Mutant IDH1 or IDH2 expressing cells may also have an impaired ability to neutralize reactive oxygen species (ROS). This is because the conversion of alpha-ketoglutarate to D-2HG consumes one molecule of NADPH, which will lead to depletion of NADPH and less formation of GSH (glutathione) which is an important antioxidant. Consequently IDH mutant cells remove free radicals less effectively, which can lead to DNA mutations [26, 41]. The increased ROS in *IDH1* or *IDH2* mutant tumours can also influence the sensitivity to chemo- and radiotherapy. This has already been shown in gliomas and can also be of importance in other tumour types with *IDH1* or *IDH2* mutations [42-45].

IDH1 and *IDH2* mutations in enchondroma and chondrosarcoma

In enchondromas with an *IDH1* or *IDH2* mutation DNA hyper methylation is observed [9] and elevated levels of 2HG were shown in mutated cartilaginous tumours compared to wild type tumours [22].

To assess the influence of the *IDH1* or *IDH2* mutation on the development of enchondroma, mesenchymal stem cells (MSCs) have been used as a model system, because the hypothesis is that enchondromas and chondrosarcomas result from altered differentiation of MSCs during bone development. MSCs are the precursors of bone, cartilage and fat tissue and can be forced to differentiate into these specific lineages in vitro, by adding

stimulating growth factors. Both D2HG addition, as well as the introduction of an *IDH1 R132C* mutation have been found to cause a block in osteogenic differentiation and increased differentiation towards the chondrogenic lineage in human MSCs [46, 47]. Also in zebra fish D2HG impairs the development of vertebrate rings, indicating a block in osteogenic differentiation during skeletogenesis [47]. The introduction of *IDH1 R132C* did not only cause an increase in cartilage differentiation, but also resulted in abnormal cartilage formation [46]. This may have something to do with the PHD enzymes involved in collagen maturation, which are affected by the increased D2HG levels [37].

Recently Hirata et al described a conditionally inducible Col2a1 specific *IDH1 132* knock-in mouse model, which showed multiple enchondroma-like cartilage lesions adjacent to the growth plates of the knee after three months. Also after six months the same lesions were still present, without significant differences in size or amount. Cartilage lesions showed patchy expression of Col10a1 indicating deregulated chondrocyte differentiation. Chondrocytes cultured from the lesions of those mice showed increased expression of hedgehog target genes and genes expressed by hypertrophic chondrocytes [48]. A defect in differentiation as well as a hyper methylation phenotype was also observed in *IDH2 R172* knock in murine 10T1/2 mesenchymal progenitor cells. Moreover, subcutaneous injection of these cells in mice led to the formation of undifferentiated sarcomas in vivo indicating that *IDH2 R172* also has tumour promoting potential [49].

In chondrosarcoma cell lines, and in primary tumours of patients with enchondroma and chondrosarcoma with an *IDH1* or *IDH2* mutation, increased levels of DNA methylation are observed compared to wild type cell lines. This hypermethylation was only found at CpG islands, but not at other regions [49, 50].

Increased levels of D2HG were found in chondrosarcoma cell lines with *IDH1* or *IDH2* mutations compared to wild type cell lines and could be diminished by treatment with a mutant specific IDH1 inhibitor (AGI-5198), however no effect was observed on cell viability, proliferation and migration [50, 51]. Likewise, cells cultured for longer periods in the presence of AGI-5198 did not show any reduction in cell viability [50]. Only after treatment with extremely high concentrations, an effect was observed on colony formation, migration and apoptosis [51].

The minor effects observed after inhibition of IDH1 mutant function and consequently D2HG levels, suggests that mutations in *IDH1* and *IDH2* are

not essential for the proliferation and metastasis capacity of malignant chondrosarcoma cells and that *IDH1* or *IDH2* mutations are no driver mutations anymore once progression towards malignant chondrosarcoma is completed.

COL2A1 mutations

Collagen type II alpha 1 (*COL2A1*) mutations have been identified in 37% [52] and 19.3% of central chondrosarcomas and 31.7% of enchondromas [53]. Type II Collagen is the major extracellular component of cartilage and is important for the strength and flexibility of the tissue. Mutations in the *COL2A1* gene can lead to abnormalities in the formation of collagen. Immunohistochemical analysis revealed that only a small part of chondrosarcomas showed complete loss of the Col2a1 protein, while most others still showed focal staining. Mutations in *COL2A1* are associated with several developmental disorders called type II collagenopathies; however no increased incidence of enchondromas or chondrosarcomas is seen in these disorders. Also in mice expressing a mutant *COL2A1* no increased chondrocyte proliferation was observed. However, a disorganized growth plate and an aberrant structure of the collagen network was present [54]. Down regulation of Col2A1 in cultured mouse chondrocytes resulted in an up regulation of Collagen I. Also Hedgehog signalling was affected by knock down of Col2A1, which resulted in a defect in chondrocyte differentiation [55]. Whether mutations in *COL2A1* are drivers of chondrosarcoma formation is still to be determined, since proof of principle studies have not yet been performed.

Dysregulation of the hedgehog pathway

The Indian hedgehog (IHH) pathway is important for bone development. Chondrocytes located in the growth plate undergo differentiation, resulting in longitudinal growth of the bone. This process is regulated by Indian hedgehog and parathyroid hormone-like hormone (PTHLH) in a negative feedback loop. Chondrocytes are differentiating towards hypertrophic chondrocytes after which they will be replaced by osteoblasts. Pre-hypertrophic chondrocytes produce IHH, stimulating proliferation of chondrocytes and stimulating the cells of the perichondrium to produce PTHLH. PTHLH in its turn inhibits IHH and thereby terminal differentiation of chondrocytes. Constitutive activation of the hedgehog pathway will result

in a failure to induce terminal differentiation of growth plate chondrocytes, which can result in the development of enchondromas [56].

Expression studies showed that the hedgehog pathway members were higher expressed in enchondromas as well as in central chondrosarcomas maintaining tumour cells in a less differentiated, but proliferative state. Levels of expression were variable, but there was no correlation with histological grade [57]. Blocking of the hedgehog pathway with triparanol or IPI-926 resulted in reduced tumour volume in chondrosarcoma xenografts [57, 58], while cyclopamine did not decrease proliferation of chondrosarcoma cell lines [59] suggesting that the tumour microenvironment may play a role in this pathway. Also the potency of the different compounds used in these studies can be different.

Patients with enchondromatosis show mutations in one of the genes involved in the IHH pathway in 8% of the cases [60, 61]. Also mice expressing a mutant parathyroid hormone-like hormone receptor specifically in chondrocytes show a deregulated growth plate and formation of cartilage lesions [61].

Hedgehog signalling is activated by binding of hedgehog ligand to the Patched receptor, releasing the Smoothened receptor and thereby Gli transcription factors are no longer inhibited and can travel to the nucleus to activate transcription. Similar to the mutant PTHLH receptor expressing mice, mice constitutively expressing Gli2 in their chondrocytes develop cartilage lesions [61].

Primary cilia have been shown to be involved in IHH signalling and to regulate its activity [61-63]. Primary cilia are also important for the columnar organization of the growth plate [64]. It has been shown that chondrosarcoma cells express less cilia, compared to normal chondrocytes, which correlated with deregulated hedgehog signalling [65]. Inhibition of the hedgehog pathway in enchondromas and central chondrosarcomas can be a possible therapeutic strategy to treat patients with inoperable or metastatic disease. A phase II clinical trial was conducted investigating the efficacy of GDC-0449 Smoothened inhibitor in 45 patients with progressive advanced chondrosarcoma, however only modest activity was observed, with stable disease in only ten patients with grade I tumours after six months [66].

Recently mutations in IHH genes have been identified in 18% of chondrosarcomas using whole exome sequencing [52]. It would be

interesting to see whether these patients will respond better to treatment with hedgehog pathway inhibitors, making it possible to preselect patients for treatment.

P53 and pRb pathway alterations in the progression from low to high grade chondrosarcoma

Mutations in the *TP53* gene are the most frequently observed mutations in cancer, often associated with a more aggressive phenotype and worse prognosis [67, 68]. Mutations in *TP53* can either lead to loss of function or gain of function [69]. Wild type p53 has important functions in controlling cell proliferation and apoptosis, and loss of function mutations lead to cancer as was proven by a *TP53* knock out mouse model [70]. However, point mutations in *TP53* will lead to pathogenic accumulation of p53 in the nucleus, and mutant *TP53* knock-in mice present with highly invasive tumours [71, 72].

In chondrosarcoma *TP53* mutations are observed in 20% of the cases and associated with more malignant tumours and a higher histological grade. Also increased nuclear p53 expression is correlated with a higher histological grade [52, 73, 74] as well as with a worse survival [75]. Mice constitutively expressing Gli, crossed with p53 knock out mice, developed low grade chondrosarcomas, indicating that deregulation of p53 is important in the progression of enchondromas to chondrosarcoma [76].

The retinoblastoma (pRb) pathway is important in the control of the cell cycle. It is the governor of the restriction point transition (R-Point), and decides whether to progress to S-phase or retreat to G0. Mutations are found in several cancer types; however in chondrosarcoma no mutations in the RB gene have been identified [74]. Also pRb expression was normal in chondrosarcomas [77].

A consistent genomic alteration identified in central chondrosarcomas is the amplification of 12q13 [74, 78]. In this region both *cyclin dependent kinase 4 (CDK4)* and *mouse double minute 2 homolog (MDM2)* are located, which play important roles in the pRb and p53 pathways. CDK4 forms a complex with cyclin D1 and together they control the transition through the G1 restriction point by inactivating pRb. Consistent with the 12q13 amplification observed in a subset of chondrosarcomas, CDK4 and Cyclin D1 expression has been shown to be upregulated in 62-73% of the high grade chondrosarcomas [78, 79]. Furthermore inhibition of CDK4 resulted

in a decrease in cell proliferation in chondrosarcoma cell lines [79]. CDKN2A/p16 is a negative regulator of the CDK4-cyclin D1 complex and is down regulated in 20% of chondrosarcomas, which also correlated with histological grade [80]. Moreover, in 28% of chondrosarcomas mutations in *CDKN2A* are observed [52]. However no loss of *CDKN2A*/p16 was found in enchondromas [80-82]. Decreased cell growth, but not apoptosis was observed in cells were CDKN2A/p16 was re-expressed [79]. MDM2 is a negative regulator of p53, targeting it for proteosomal degradation. MDM2 has been shown to be overexpressed in 34% of high grade chondrosarcomas indicating that p53 is degraded with a higher rate in these tumours [79].

The combined incidence of mutations in either the p53 or pRb pathways in high grade chondrosarcomas is 96% [79]. This suggests that these pathways are important in the progression of chondrosarcoma towards a more malignant histological phenotype.

Apoptosis and pro-survival pathways implicated in chondrosarcoma

A downstream target in the hedgehog pathway is Bcl-2, which is upregulated in the growth plate and inhibits differentiation of proliferating chondrocytes after binding of PTHrP to its receptor. In conventional chondrosarcoma Bcl-2 is highly expressed [83, 84] as well as in rare chondrosarcoma subtypes [85].

By up regulating anti-apoptotic proteins of the Bcl-2 family, chondrosarcoma cells can cause resistance to chemotherapy, which is a major problem in the treatment of these tumours. This has been shown in a cell model where chondrosarcoma cells could be sensitized to chemotherapy when treated in combination with a Bcl-2 family inhibitor (ABT-737) [85, 86].

Another protein highly expressed in chondrosarcoma is Survivin [87-89]. Survivin plays an important role in the cell cycle as well as in the apoptotic pathway. Furthermore it is expressed at very low levels in normal tissue, making it an ideal therapeutic target [90]. In preclinical studies a small molecule Survivin inhibitor (YM155) was shown to decrease cell viability in chondrosarcoma cell lines, which was dependent on *TP53* mutation status [91]. Also down regulation of Survivin using siRNAs showed decreased viability and increased sensitivity towards chemotherapeutics [87].

Several studies have shown the importance of the Akt-Pi3K-mTOR pathway in chondrosarcoma. In 69% of conventional chondrosarcoma and 44% of dedifferentiated chondrosarcoma S6 was phosphorylated indicating an active PI3K/mTOR activity. Also treatment with a dual PI3K/mTOR inhibitor (Bez235) resulted in reduced cell viability in chondrosarcoma cell lines and inhibition of tumour growth in a mouse xenografts model [92]. In a rat chondrosarcoma model treatment with everolimus resulted in inhibition of tumour growth [93].

Peripheral chondrosarcoma

EXT1 and *EXT2* mutations in osteochondroma and peripheral chondrosarcoma

Multiple osteochondromas (MO) is an autosomal dominant condition in which mutations in tumour suppressor genes *Exostosin glycosyltransferase 1* or *Exostosin glycosyltransferase 2* (*EXT1* and *EXT2*) have been identified [94-96]. Patients with this disease develop osteochondromas at the surface of their bones and show heterozygous germline *EXT1* or *EXT2* mutations, with loss of the wildtype allele in the osteochondroma cartilaginous cap at least in a subset of the cases [97]. Also homozygous deletions in *EXT1* have been identified in a subset of sporadic osteochondromas [98]. Both hereditary and sporadic osteochondromas can progress towards peripheral chondrosarcoma (see figure 1). This occurs in ~1% of sporadic and up to 5% of hereditary osteochondromas [99].

Exostosin-1 and exostosin-2 are both type II glycosyltransferases which function in the biosynthesis of heparan sulfate chains of proteoglycans [100]. Proteoglycans are molecules that can regulate the signalling of several growth factors in many different ways. They can act as receptors or coreceptors in signalling pathways, regulate receptor trafficking by endo- and exocytosis, facilitate ligand secretion, function as a signal to other cells, or act by presenting a ligand to a receptor. In addition, they can regulate the structure of the extracellular matrix by providing a signalling gradient to ligands [101]. In the growth plate, heparan sulfate is important for the establishment of a gradient of IHH (as discussed above). When the biosynthesis of heparan sulfate is deregulated, the diffusion of IHH signalling is disturbed. This will have consequences for the polar organization of growth plate chondrocytes and the formation of the bony collar, leading to the formation of an osteochondroma [64].

In a conditional inducible mouse model, where both *Ext1* alleles were inactivated specifically in chondrocytes, the development of osteochondromas was observed, mimicking the human situation. The osteochondromas consisted of a mixture of wild type and mutated cells indicating that cells need normal heparan sulfate synthesis to survive [102]. In another mouse model with a heterozygous loss of both *Ext1* and *Ext2* a decrease in heparan sulfate was observed as well as more exostoses compared to mice with heterozygous loss of *Ext1* or *Ext2* [103]. Zebrafish with homozygous loss of *Ext2* in their chondrocytes show osteochondroma-like lesions [104]. In addition, they show altered mesenchymal stem cell differentiation [105].

Osteochondromas can progress into peripheral chondrosarcomas in a small percentage of cases. It has been shown that the majority of peripheral chondrosarcomas has at least one wild type allele for *EXT1* and *EXT2*, indicating that functional EXT is required for cells to survive and to undergo malignant progression [106]. Most likely additional genetic alterations are needed in order to progress to peripheral chondrosarcoma and they probably take place in the *EXT1* and *EXT2* wild type cells.

p53 and pRb pathway alterations

In peripheral chondrosarcoma pRb and p53 pathway alterations are also found in the majority of the cases, especially in tumours with higher histological grade. A mouse model with conditional inducible *Ext1* inactivation specifically in chondrocytes combined with conditional loss of *Trp53* or Ink4a/Arf showed peripheral chondrosarcoma development from Ext-null and Ext mutated cells, mimicking the human situation. Also loss of primary cilia and polar organization was observed in these lesions. This indicates that an alteration in one of the pathways regulating the cell cycle in addition to the *EXT* mutation is needed for the development of peripheral chondrosarcoma from an osteochondroma [107].

Mesenchymal chondrosarcoma: HEY1-NCOA2 fusion

Mesenchymal chondrosarcoma is characterized by a specific gene fusion between *HEY1* (*Hes-Related Family BHLH Transcription Factor with YRPW Motif 1*) and *NCOA2* (*Nuclear Receptor Coactivator 2*) [108]. *HEY1* is a basic helix-loop-helix transcription factor and downstream of the Notch signalling pathway [109, 110]. In rhabdomyosarcoma inhibition of Hey1 resulted in

downregulation of Notch1 and myogenic differentiation [111]. *NCOA2*, also known as *SRC2* (steroid receptor co-activator 2), is a nuclear hormone receptor co-activator, important for chromatin remodelling [112]. Overexpression of NCOA2 was found in prostate cancer and was correlated with metastatic disease [113]. Rearrangements involving *NCOA2* have been identified in other sarcomas as well [114, 115]. The exact mechanism by which the *HEY1-NCOA2* fusion leads to the formation of mesenchymal chondrosarcoma is currently unknown.

Clear cell chondrosarcoma

Clear cell chondrosarcomas are rare chondrosarcomas subtypes, and limited genetic defects have been identified in these tumours. Array-CGH analysis was performed, identifying hemizygous loss of the *CDKN2A*/p16 locus in four out of twelve clear cell chondrosarcomas. However loss of p16 protein expression has been identified in 95% of cases, indicating that aberrations in the pRb pathway are important for the development and/or progression of this tumour type [116].

Summary/conclusion

Treatment of chondrosarcoma patients is mainly by surgery, since these tumours are resistant to conventional chemo and radiotherapy. Therefore it is important to understand the molecular pathways involved in the development and progression of chondrosarcoma. Chondrosarcomas in the skull or spine present a challenge to remove surgically due to their location. Especially for these patients new therapeutic options are needed. In table 1 the most important genetic alterations and pathways that are deregulated in chondrosarcoma are listed. The most frequently observed subtype is central chondrosarcoma, which is characterized by mutations in *IDH1* or *IDH2* in 38-70 % of the cases (see figure 1). Preclinical research exploring the possibility of specifically inhibiting the mutant IDH1 did not lead to a decrease in cell proliferation, indicating that this will not be a therapeutic target for chondrosarcoma patients. However future studies investigating synthetic lethal approaches might provide alternative options exploiting IDH mutation induced vulnerabilities. Promising targets for therapy include the Bcl-2 family members and mTOR, which are highly active in chondrosarcoma. Currently a phase II clinical trial investigating the effect of

mTOR inhibition in combination with cyclophosphamide has started, of which results will be awaited with great interest.

Targeting the IHH pathway may offer another strategy to treat chondrosarcoma patients, since this pathway is deregulated in chondrosarcoma, although a phase II clinical trial did not show promising results. However, recently mutations in IHH genes have been identified in 18% of chondrosarcomas using whole exome sequencing, and perhaps patients with these mutations will benefit from treatment. In peripheral chondrosarcoma *EXT1* and *EXT2* mutations have been found in peripheral chondrosarcoma, influencing the IHH pathway as well, however in contrast to conventional chondrosarcoma this pathway is not activated in peripheral chondrosarcoma making it not suitable for treatment.

Future research will focus on identification of additional molecular pathways that are involved in the development and progression of chondrosarcoma to find therapeutic targets to treat patients with unresectable chondrosarcoma.

Table 1. Genetic alterations and deregulated pathways found in different subtypes of chondrosarcoma. CS= Chondrosarcoma

	Gene/Pathway	***Subtype***	***Reference***
Genetic alterations			
	IDH1 or *IDH2*	Enchondroma Central CS Dedifferentiated CS Periosteal CS	9,19,22,23
	EXT1 or *EXT2*	Osteochondroma Peripheral CS	98 106
	COL2A1	Enchondroma Central CS	52,53
	IHH pathway members	Central CS Peripheral CS	52
	HEY1-NCOA2 fusion	Mesenchymal CS	108
	TP53	Central CS Peripheral CS	52,73,74
	CDKN2A	Central CS Peripheral CS	52
Pathway deregulation			
	p53 and pRB pathway	Central CS Peripheral CS Clear cell CS	79 107 116

	Bcl-2 family members	Central CS Dedifferentiated CS Mesenchymal CS Clear cell CS	83,84,85
	Survivin	Central CS Dedifferentiated CS Mesenchymal CS Clear cell CS	87,88,89,91
	Pi3K/mTOR	Central CS Peripheral CS Dedifferentiated CS	92

References

1. Fletcher CDM, Bridge JA, Hogendoorn PC, Mertens F: WHO Classification of Tumours of Soft Tissue and Bone.2013.
2. Katonis P, Alpantaki K, Michail K, Lianoudakis S, Christoforakis Z, Tzanakakis G, Karantanas A: Spinal chondrosarcoma: a review. Sarcoma 2011, 2011:378957.
3. Flanagan AM, Yamaguchi T: Chordoma. In WHO classification of tumours of soft tissue and bone. Volume 4th edition2013: 328-329
4. Hogendoorn PCW, Bovee JVMG, Nielsen GP: Chondrosarcoma (grades I-III), including primary and secondary variants and periosteal chondrosarcoma. In WHO Classification of Tumours of Soft Tissue and Bone. Edited by Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F2013: 264-268
5. Inwards C, Hogendoorn PCW: Dedifferentiated chondrosarcoma. In WHO Classification of Tumours of Soft Tissue and Bone. Edited by Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F2013: 269-270
6. McCarthy EF, Hogendoorn PCW: Clear Cell Chondrosarcoma. In WHO classification of Tumours of Soft Tissue and Bone. Volume 42013: 273-274
7. Nakashima Y, de Pinieux G, Ladanyi M: Mesenchymal chondrosarcoma. In WHO Classification of Tumours of Soft Tissue and Bone. Edited by Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F2013: 271-272
8. Bloch O, Parsa AT: Skull base chondrosarcoma: evidence-based treatment paradigms. NeurosurgClinNAm 2013, 24:89-96.
9. Pansuriya TC, van ER, d'Adamo P, van Ruler MA, Kuijjer ML, Oosting J, Cleton-Jansen AM, van Oosterwijk JG, Verbeke SL, Meijer D, et al: Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. NatGenet 2011, 43:1256-1261.
10. Verdegaal SH, Bovee JV, Pansuriya TC, Grimer RJ, Ozger H, Jutte PC, San JM, Biau DJ, van der Geest IC, Leithner A, et al: Incidence, predictive factors, and prognosis of chondrosarcoma in patients with Ollier disease and Maffucci syndrome: an international multicenter study of 161 patients. Oncologist 2011, 16:1771-1779.
11. Gelderblom H, Hogendoorn PCW, Dijkstra SD, van Rijswijk CS, Krol AD, Taminiau AH, Bovee JV: The clinical approach towards chondrosarcoma. Oncologist 2008, 13:320-329.

12. Bjornsson J, McLeod RA, Unni KK, Ilstrup DM, Pritchard DJ: Primary chondrosarcoma of long bones and limb girdles. Cancer 1998, 83:2105-2119.
13. 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.
14. Italiano A, Mir O, Cioffi A, Palmerini E, Piperno-Neumann S, Perrin C, Chaigneau L, Penel N, Duffaud F, Kurtz JE, et al: Advanced chondrosarcomas: role of chemotherapy and survival. AnnOncol 2013, 24:2916-2922.
15. Grimer RJ, Gosheger G, Taminiau A, Biau D, Matejovsky Z, Kollender Y, San-Julian M, Gherlinzoni F, Ferrari C: Dedifferentiated chondrosarcoma: prognostic factors and outcome from a European group. EurJCancer 2007, 43:2060-2065.
16. Dantonello TM, Int-Veen C, Leuschner I, Schuck A, Furtwaengler R, Claviez A, Schneider DT, Klingebiel T, Bielack SS, Koscielniak E: Mesenchymal chondrosarcoma of soft tissues and bone in children, adolescents, and young adults: experiences of the CWS and COSS study groups. Cancer 2008, 112:2424-2431.
17. Frezza AM, Cesari M, Baumhoer D, Biau D, Bielack S, Campanacci DA, Casanova J, Esler C, Ferrari S, Funovics PT, et al: Mesenchymal chondrosarcoma: prognostic factors and outcome in 113 patients. A European Musculoskeletal Oncology Society study. EurJCancer 2015, 51:374-381.
18. Huvos AG, Rosen G, Dabska M, Marcove RC: Mesenchymal chondrosarcoma. A clinicopathologic analysis of 35 patients with emphasis on treatment. Cancer 1983, 51:1230-1237.
19. Cleven AH, Zwartkruis E, Hogendoorn PC, Kroon HM, Briaire-de BI, Bovee JV: Periosteal chondrosarcoma: a histopathological and molecular analysis of a rare chondrosarcoma subtype. Histopathology 2015, 67:483-490.
20. 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.
21. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, et al: An integrated genomic analysis of human glioblastoma multiforme. Science 2008, 321:1807-1812.
22. Amary MF, Bacsi K, Maggiani F, Damato S, Halai D, Berisha F, Pollock R, O'Donnell P, Grigoriadis A, Diss T, et al: IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. JPathol 2011, 224:334-343.
23. Damato S, Alorjani M, Bonar F, McCarthy SW, Cannon SR, O'Donnell P, Tirabosco R, Amary MF, Flanagan AM: IDH1 mutations are not found in cartilaginous tumours other than central and periosteal chondrosarcomas and enchondromas. Histopathology 2012, 60:363-365.
24. Arai M, Nobusawa S, Ikota H, Takemura S, Nakazato Y: Frequent IDH1/2 mutations in intracranial chondrosarcoma: a possible diagnostic clue for its differentiation from chordoma. Brain Tumor Pathol 2012, 29:201-206.
25. Kanamori H, Kitamura Y, Kimura T, Yoshida K, Sasaki H: Genetic characterization of skull base chondrosarcomas. JNeurosurg 2015, 123:1036-1041.

26. Molenaar RJ, Radivoyevitch T, Maciejewski JP, van Noorden CJ, Bleeker FE: The driver and passenger effects of isocitrate dehydrogenase 1 and 2 mutations in oncogenesis and survival prolongation. BiochimBiophysActa 2014, 1846:326-341.
27. Cairns RA, Mak TW: Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. Cancer Discov 2013, 3:730-741.
28. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, et al: Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 2009, 462:739-744.
29. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, et al: Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell 2011, 19:17-30.
30. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y: Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 2010, 466:1129-1133.
31. Kriaucionis S, Heintz N: The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 2009, 324:929-930.
32. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A: Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 2009, 324:930-935.
33. Dawlaty MM, Breiling A, Le T, Barrasa MI, Raddatz G, Gao Q, Powell BE, Cheng AW, Faull KF, Lyko F, Jaenisch R: Loss of Tet enzymes compromises proper differentiation of embryonic stem cells. DevCell 2014, 29:102-111.
34. Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA, Rose NR, Leung IK, Li XS, Woon EC, Yang M, et al: The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. EMBO Rep 2011, 12:463-469.
35. Lu C, Thompson CB: Metabolic regulation of epigenetics. Cell Metab 2012, 16:9-17.
36. Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, Losman JA, Joensuu P, Bergmann U, Gross S, et al: Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature 2012, 483:484-488.
37. Sasaki M, Knobbe CB, Itsumi M, Elia AJ, Harris IS, Chio II, Cairns RA, McCracken S, Wakeham A, Haight J, et al: D-2-hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function. Genes Dev 2012, 26:2038-2049.
38. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von KA, Hebestreit HF, Mukherji M, Schofield CJ, et al: Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 2001, 292:468-472.
39. Heuser M, Araujo Cruz MM, Goparaju R, Chaturvedi A: Enigmas of IDH mutations in hematology/oncology. ExpHematol 2015, 43:685-697.
40. Parker SJ, Metallo CM: Metabolic consequences of oncogenic IDH mutations. PharmacolTher 2015, 152:54-62.
41. Sosa V, Moline T, Somoza R, Paciucci R, Kondoh H, Lleonart ME: Oxidative stress and cancer: an overview. Ageing ResRev 2013, 12:376-390.
42. Houillier C, Wang X, Kaloshi G, Mokhtari K, Guillevin R, Laffaire J, Paris S, Boisselier B, Idbaih A, Laigle-Donadey F, et al: IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. Neurology 2010, 75:1560-1566.

43. Li S, Chou AP, Chen W, Chen R, Deng Y, Phillips HS, Selfridge J, Zurayk M, Lou JJ, Everson RG, et al: Overexpression of isocitrate dehydrogenase mutant proteins renders glioma cells more sensitive to radiation. NeuroOncol 2013, 15:57-68.
44. Molenaar RJ, Botman D, Smits MA, Hira VV, van Lith SA, Stap J, Henneman P, Khurshed M, Lenting K, Mul AN, et al: Radioprotection of IDH1-Mutated Cancer Cells by the IDH1-Mutant Inhibitor AGI-5198. Cancer Res 2015, 75:4790-4802.
45. Okita Y, Narita Y, Miyakita Y, Ohno M, Matsushita Y, Fukushima S, Sumi M, Ichimura K, Kayama T, Shibui S: IDH1/2 mutation is a prognostic marker for survival and predicts response to chemotherapy for grade II gliomas concomitantly treated with radiation therapy. IntJOncol 2012, 41:1325-1336.
46. Jin Y, Elalaf H, Watanabe M, Tamaki S, Hineno S, Matsunaga K, Woltjen K, Kobayashi Y, Nagata S, Ikeya M, et al: Mutant IDH1 Dysregulates the Differentiation of Mesenchymal Stem Cells in Association with Gene-Specific Histone Modifications to Cartilage- and Bone-Related Genes. PLoSOne 2015, 10:e0131998.
47. Suijker J, Baelde HJ, Roelofs H, Cleton-Jansen AM, Bovee JV: The oncometabolite D-2-hydroxyglutarate induced by mutant IDH1 or -2 blocks osteoblast differentiation in vitro and in vivo. Oncotarget 2015, 6:14832-14842.
48. Hirata M, Sasaki M, Cairns RA, Inoue S, Puviindran V, Li WY, Snow BE, Jones LD, Wei Q, Sato S, et al: Mutant IDH is sufficient to initiate enchondromatosis in mice. ProcNatlAcadSciUSA 2015, 112:2829-2834.
49. Lu C, Venneti S, Akalin A, Fang F, Ward PS, Dematteo RG, Intlekofer AM, Chen C, Ye J, Hameed M, et al: Induction of sarcomas by mutant IDH2. Genes Dev 2013, 27:1986-1998.
50. Suijker J, Oosting J, Koornneef A, Struys EA, Salomons GS, Schaap FG, Waaijer CJ, Wijers-Koster PM, Briaire-de Bruijn IH, Haazen L, et al: Inhibition of mutant IDH1 decreases D-2-HG levels without affecting tumorigenic properties of chondrosarcoma cell lines. Oncotarget 2015, 6:12505-12519.
51. Li L, Paz AC, Wilky BA, Johnson B, Galoian K, Rosenberg A, Hu G, Tinoco G, Bodamer O, Trent JC: Treatment with a Small Molecule Mutant IDH1 Inhibitor Suppresses Tumorigenic Activity and Decreases Production of the Oncometabolite 2-Hydroxyglutarate in Human Chondrosarcoma Cells. PLoSOne 2015, 10:e0133813.
52. Tarpey PS, Behjati S, Cooke SL, Van LP, Wedge DC, Pillay N, Marshall J, O'Meara S, Davies H, Nik-Zainal S, et al: Frequent mutation of the major cartilage collagen gene COL2A1 in chondrosarcoma. NatGenet 2013, 45:923-926.
53. Totoki Y, Yoshida A, Hosoda F, Nakamura H, Hama N, Ogura K, Yoshida A, Fujiwara T, Arai Y, Toguchida J, et al: Unique mutation portraits and frequent COL2A1 gene alteration in chondrosarcoma. Genome Res 2014, 24:1411-1420.
54. Arita M, Fertala J, Hou C, Steplewski A, Fertala A: Mechanisms of aberrant organization of growth plates in conditional transgenic mouse model of spondyloepiphyseal dysplasia associated with the R992C substitution in collagen II. AmJPathol 2015, 185:214-229.

55. Xin W, Heilig J, Paulsson M, Zaucke F: Collagen II regulates chondroycte integrin expression profile and differentiation. ConnectTissue Res 2015, 56:307-314.
56. Bovee JV, Hogendoorn PC, Wunder JS, Alman BA: Cartilage tumours and bone development: molecular pathology and possible therapeutic targets. Nat Rev Cancer 2010, 10:481-488.
57. Tiet TD, Hopyan S, Nadesan P, Gokgoz N, Poon R, Lin AC, Yan T, Andrulis IL, Alman BA, Wunder JS: Constitutive hedgehog signaling in chondrosarcoma up-regulates tumor cell proliferation. AmJPathol 2006, 168:321-330.
58. Campbell VT, Nadesan P, Ali SA, Wang CY, Whetstone H, Poon R, Wei Q, Keilty J, Proctor J, Wang LW, et al: Hedgehog pathway inhibition in chondrosarcoma using the smoothened inhibitor IPI-926 directly inhibits sarcoma cell growth. MolCancer Ther 2014, 13:1259-1269.
59. Schrage YM, Hameetman L, Szuhai K, Cleton-Jansen AM, Taminiau AH, Hogendoorn PC, Bovee JV: Aberrant heparan sulfate proteoglycan localization, despite normal exostosin, in central chondrosarcoma. AmJPathol 2009, 174:979-988.
60. Couvineau A, Wouters V, Bertrand G, Rouyer C, Gerard B, Boon LM, Grandchamp B, Vikkula M, Silve C: PTHR1 mutations associated with Ollier disease result in receptor loss of function. HumMolGenet 2008, 17:2766-2775.
61. Hopyan S, Gokgoz N, Poon R, Gensure RC, Yu C, Cole WG, Bell RS, Juppner H, Andrulis IL, Wunder JS, Alman BA: A mutant PTH/PTHrP type I receptor in enchondromatosis. NatGenet 2002, 30:306-310.
62. Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF: Vertebrate Smoothened functions at the primary cilium. Nature 2005, 437:1018-1021.
63. Goetz SC, Anderson KV: The primary cilium: a signalling centre during vertebrate development. NatRevGenet 2010, 11:331-344.
64. de Andrea CE, Wiweger M, Prins F, Bovee JV, Romeo S, Hogendoorn PC: Primary cilia organization reflects polarity in the growth plate and implies loss of polarity and mosaicism in osteochondroma. Lab Invest 2010, 90:1091-1101.
65. Ho L, Ali SA, Al-Jazrawe M, Kandel R, Wunder JS, Alman BA: Primary cilia attenuate hedgehog signalling in neoplastic chondrocytes. Oncogene 2013, 32:5388-5396.
66. Italiano A, Le CA, Bellera C, Piperno-Neumann S, Duffaud F, Penel N, Cassier P, Domont J, Takebe N, Kind M, et al: GDC-0449 in patients with advanced chondrosarcomas: a French Sarcoma Group/US and French National Cancer Institute Single-Arm Phase II Collaborative Study. AnnOncol 2013, 24:2922-2926.
67. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, et al: Mutational landscape and significance across 12 major cancer types. Nature 2013, 502:333-339.
68. Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, Stuart JM: The Cancer Genome Atlas Pan-Cancer analysis project. NatGenet 2013, 45:1113-1120.
69. Soussi T, Wiman KG: TP53: an oncogene in disguise. Cell DeathDiffer 2015, 22:1239-1249.

70. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Jr., Butel JS, Bradley A: Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992, 356:215-221.
71. Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, Crowley D, Jacks T: Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell 2004, 119:847-860.
72. Lang GA, Iwakuma T, Suh YA, Liu G, Rao VA, Parant JM, Valentin-Vega YA, Terzian T, Caldwell LC, Strong LC, et al: Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. Cell 2004, 119:861-872.
73. Terek RM, Healey JH, Garin-Chesa P, Mak S, Huvos A, Albino AP: p53 mutations in chondrosarcoma. DiagnMolPathol 1998, 7:51-56.
74. Yamaguchi T, Toguchida J, Wadayama B, Kanoe H, Nakayama T, Ishizaki K, Ikenaga M, Kotoura Y, Sasaki MS: Loss of heterozygosity and tumor suppressor gene mutations in chondrosarcomas. Anticancer Res 1996, 16:2009-2015.
75. Oshiro Y, Chaturvedi V, Hayden D, Nazeer T, Johnson M, Johnston DA, Ordonez NG, Ayala AG, Czerniak B: Altered p53 is associated with aggressive behavior of chondrosarcoma: a long term follow-up study. Cancer 1998, 83:2324-2334.
76. Ho L, Stojanovski A, Whetstone H, Wei QX, Mau E, Wunder JS, Alman B: Gli2 and p53 cooperate to regulate IGFBP-3- mediated chondrocyte apoptosis in the progression from benign to malignant cartilage tumors. Cancer Cell 2009, 16:126-136.
77. Ropke M, Boltze C, Meyer B, Neumann HW, Roessner A, Schneider-Stock R: Rb-loss is associated with high malignancy in chondrosarcoma. OncolRep 2006, 15:89-95.
78. Rozeman LB, Szuhai K, Schrage YM, Rosenberg C, Tanke HJ, Taminiau AH, Cleton-Jansen AM, Bovee JV, Hogendoorn PC: Array-comparative genomic hybridization of central chondrosarcoma: identification of ribosomal protein S6 and cyclin-dependent kinase 4 as candidate target genes for genomic aberrations. Cancer 2006, 107:380-388.
79. Schrage YM, Lam S, Jochemsen AG, Cleton-Jansen AM, Taminiau AH, Hogendoorn PC, Bovee JV: Central chondrosarcoma progression is associated with pRb pathway alterations: CDK4 down-regulation and p16 overexpression inhibit cell growth in vitro. JCell MolMed 2009, 13:2843-2852.
80. van Beerendonk HM, Rozeman LB, Taminiau AH, Sciot R, Bovee JV, Cleton-Jansen AM, Hogendoorn PC: Molecular analysis of the INK4A/INK4A-ARF gene locus in conventional (central) chondrosarcomas and enchondromas: indication of an important gene for tumour progression. JPathol 2004, 202:359-366.
81. Asp J, Brantsing C, Benassi MS, Inerot S, Sangiorgi L, Picci P, Lindahl A: Changes in p14(ARF) do not play a primary role in human chondrosarcoma tissues. IntJCancer 2001, 93:703-705.
82. Asp J, Inerot S, Block JA, Lindahl A: Alterations in the regulatory pathway involving p16, pRb and cdk4 in human chondrosarcoma. JOrthopRes 2001, 19:149-154.
83. Bovee JV, van den Broek LJ, Cleton-Jansen AM, Hogendoorn PC: Up-regulation of PTHrP and Bcl-2 expression characterizes the progression of

osteochondroma towards peripheral chondrosarcoma and is a late event in central chondrosarcoma. Lab Invest 2000, 80:1925-1934.
84. Rozeman LB, Hameetman L, Cleton-Jansen AM, Taminiau AH, Hogendoorn PC, Bovee JV: Absence of IHH and retention of PTHrP signalling in enchondromas and central chondrosarcomas. JPathol 2005, 205:476-482.
85. van Oosterwijk JG, Meijer D, van Ruler MA, van den Akker BE, Oosting J, Krenacs T, Picci P, Flanagan AM, Liegl-Atzwanger B, Leithner A, et al: Screening for potential targets for therapy in mesenchymal, clear cell, and dedifferentiated chondrosarcoma reveals Bcl-2 family members and TGFbeta as potential targets. AmJPathol 2013, 182:1347-1356.
86. van Oosterwijk JG, Herpers B, Meijer D, Briaire-de Bruijn IH, Cleton-Jansen AM, Gelderblom H, van de Water B, Bovee JVMG: Restoration of chemosensitivity for doxorubicin and cisplatin in chondrosarcoma in vitro: BCL-2 family members cause chemoresistance. AnnOncol 2012, 23:1617-1626.
87. Lechler P, Renkawitz T, Campean V, Balakrishnan S, Tingart M, Grifka J, Schaumburger J: The antiapoptotic gene survivin is highly expressed in human chondrosarcoma and promotes drug resistance in chondrosarcoma cells in vitro. BMCCancer 2011, 11:120.
88. Machado I, Giner F, Mayordomo E, Carda C, Navarro S, Llombart-Bosch A: Tissue microarrays analysis in chondrosarcomas: light microscopy, immunohistochemistry and xenograft study. DiagnPathol 2008, 3 Suppl 1:S25.
89. Yang P, Wang G, Huo H, Li Q, Zhao Y, Liu Y: SDF-1/CXCR4 signaling up-regulates survivin to regulate human sacral chondrosarcoma cell cycle and epithelial-mesenchymal transition via ERK and PI3K/AKT pathway. MedOncol 2015, 32:377.
90. Altieri DC: Survivin, cancer networks and pathway-directed drug discovery. NatRevCancer 2008, 8:61-70.
91. de Jong Y, van Oosterwijk JG, Kruisselbrink AB, Briaire-de Bruijn IH, Agrogiannis G, Baranski Z, Cleven AH, Cleton-Jansen AM, van de Water B, Danen EH, Bovee JV: Targeting survivin as a potential new treatment for chondrosarcoma of bone. Oncogenesis 2016, 5:e222.
92. Zhang YX, van Oosterwijk JG, Sicinska E, Moss S, Remillard SP, van WT, Buehnemann C, Hassan AB, Demetri GD, Bovee JV, Wagner AJ: Functional profiling of receptor tyrosine kinases and downstream signaling in human chondrosarcomas identifies pathways for rational targeted therapy. ClinCancer Res 2013.
93. Perez J, Decouvelaere AV, Pointecouteau T, Pissaloux D, Michot JP, Besse A, Blay JY, Dutour A: Inhibition of chondrosarcoma growth by mTOR inhibitor in an in vivo syngeneic rat model. PLoSOne 2012, 7:e32458.
94. Cook A, Raskind W, Blanton SH, Pauli RM, Gregg RG, Francomano CA, Puffenberger E, Conrad EU, Schmale G, Schellenberg G: Genetic heterogeneity in families with hereditary multiple exostoses. AmJHumGenet 1993, 53:71-79.
95. Le Merrer M, Legeai-Mallet L, Jeannin PM, Horsthemke B, Schinzel A, Plauchu H, Toutain A, Achard F, Munnich A, Maroteaux P: A gene for hereditary multiple exostoses maps to chromosome 19p. Hum Mol Genet 1994, 3:717-722.
96. Wu YQ, Heutink P, de Vries BB, Sandkuijl LA, van den Ouweland AM, Niermeijer MF, Galjaard H, Reyniers E, Willems PJ, Halley DJ: Assignment

of a second locus for multiple exostoses to the pericentromeric region of chromosome 11. HumMolGenet 1994, 3:167-171.
97. Bovee JVMG, Cleton-Jansen AM, Wuyts W, Caethoven G, Taminiau AH, Bakker E, Van HW, Cornelisse CJ, Hogendoorn PC: EXT-mutation analysis and loss of heterozygosity in sporadic and hereditary osteochondromas and secondary chondrosarcomas. AmJHumGenet 1999, 65:689-698.
98. Hameetman L, Szuhai K, Yavas A, Knijnenburg J, van DM, van DH, Taminiau AH, Cleton-Jansen AM, Bovee JV, Hogendoorn PC: The role of EXT1 in nonhereditary osteochondroma: identification of homozygous deletions. JNatlCancer Inst 2007, 99:396-406.
99. Bovee JVMG, Heymann D, Wuyts W: Osteochondroma. In WHO classification of tumours of soft tissue and bone. 2015: 250-251
100. McCormick C, Leduc Y, Martindale D, Mattison K, Esford LE, Dyer AP, Tufaro F: The putative tumour suppressor EXT1 alters the expression of cell-surface heparan sulfate. NatGenet 1998, 19:158-161.
101. Poulain FE, Yost HJ: Heparan sulfate proteoglycans: a sugar code for vertebrate development? Development 2015, 142:3456-3467.
102. Jones KB, Piombo V, Searby C, Kurriger G, Yang B, Grabellus F, Roughley PJ, Morcuende JA, Buckwalter JA, Capecchi MR, et al: A mouse model of osteochondromagenesis from clonal inactivation of Ext1 in chondrocytes. ProcNatlAcadSciUSA 2010, 107:2054-2059.
103. Zak BM, Schuksz M, Koyama E, Mundy C, Wells DE, Yamaguchi Y, Pacifici M, Esko JD: Compound heterozygous loss of Ext1 and Ext2 is sufficient for formation of multiple exostoses in mouse ribs and long bones. Bone 2011, 48:979-987.
104. Clement A, Wiweger M, von der HS, Rusch MA, Selleck SB, Chien CB, Roehl HH: Regulation of zebrafish skeletogenesis by ext2/dackel and papst1/pinscher. PLoSGenet 2008, 4:e1000136.
105. Wiweger MI, de Andrea CE, Scheepstra KW, Zhao Z, Hogendoorn PC: Possible effects of EXT2 on mesenchymal differentiation--lessons from the zebrafish. OrphanetJRareDis 2014, 9:35.
106. de Andrea CE, Reijnders CM, Kroon HM, de JD, Hogendoorn PC, Szuhai K, Bovee JV: Secondary peripheral chondrosarcoma evolving from osteochondroma as a result of outgrowth of cells with functional EXT. Oncogene 2012, 31:1095-1104.
107. de Andrea CE, Zhu JF, Jin H, Bovee JV, Jones KB: Cell cycle deregulation and mosaic loss of Ext1 drive peripheral chondrosarcomagenesis in the mouse and reveal an intrinsic cilia deficiency. JPathol 2015, 236:210-218.
108. Wang L, Motoi T, Khanin R, Olshen A, Mertens F, Bridge J, Dal CP, Antonescu CR, Singer S, Hameed M, et al: Identification of a novel, recurrent HEY1-NCOA2 fusion in mesenchymal chondrosarcoma based on a genome-wide screen of exon-level expression data. Genes ChromosomesCancer 2012, 51:127-139.
109. Iso T, Kedes L, Hamamori Y: HES and HERP families: multiple effectors of the Notch signaling pathway. J Cell Physiol 2003, 194:237-255.
110. Weber D, Wiese C, Gessler M: Hey bHLH transcription factors. CurrTopDevBiol 2014, 110:285-315.
111. Belyea BC, Naini S, Bentley RC, Linardic CM: Inhibition of the Notch-Hey1 axis blocks embryonal rhabdomyosarcoma tumorigenesis. Clin Cancer Res 2011, 17:7324-7336.

112. Xu J, Wu RC, O'Malley BW: Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. NatRevCancer 2009, 9:615-630.
113. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, et al: Integrative genomic profiling of human prostate cancer. Cancer Cell 2010, 18:11-22.
114. Jin Y, Moller E, Nord KH, Mandahl N, Von Steyern FV, Domanski HA, Marino-Enriquez A, Magnusson L, Nilsson J, Sciot R, et al: Fusion of the AHRR and NCOA2 genes through a recurrent translocation t(5;8)(p15;q13) in soft tissue angiofibroma results in upregulation of aryl hydrocarbon receptor target genes. Genes ChromosomesCancer 2012, 51:510-520.
115. Mosquera JM, Sboner A, Zhang L, Kitabayashi N, Chen CL, Sung YS, Wexler LH, LaQuaglia MP, Edelman M, Sreekantaiah C, et al: Recurrent NCOA2 gene rearrangements in congenital/infantile spindle cell rhabdomyosarcoma. Genes ChromosomesCancer 2013, 52:538-550.
116. Meijer D, de JD, Pansuriya TC, van den Akker BE, Picci P, Szuhai K, Bovee JV: Genetic characterization of mesenchymal, clear cell, and dedifferentiated chondrosarcoma. Genes ChromosomesCancer 2012, 51:899-909.