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Chapter 1

General introduction and outline of this thesis
Primary bone cancers are a heterogeneous group of cancers that originate from the bone. They account for less than 0.2 % of all cancers (1). In the Netherlands, 228 primary bone cancers have been diagnosed in 2016 (2). This heterogeneous group of cancers differ in terms of etiology, clinical manifestation and prognosis. Here, we focus on the two most common subtypes: osteosarcoma and chondrosarcoma.

**Osteosarcoma**

Osteosarcoma is the most frequent primary malignant bone tumour. This tumour is characterized by the production of osteoid matrix. The incidence of osteosarcoma has a bimodal distribution, with the first peak around puberty and the second, smaller peak, observed above the age of 60. The incidence is 5.0 (4.6-5.6) per million 0-19 year olds (3, 4). The second peak in incidence is likely to represent a secondary malignancy, as half of the osteosarcoma patients above 60 years suffer from Paget disease of the bone (5). In addition, osteosarcoma is the most common radiation-induced sarcoma (5-7). Most primary osteosarcomas occur in the metaphysis of the long bones, in particular the distal femur (30%), the proximal tibia (15%) and the proximal humerus (15%), where bone growth is particularly active, especially at the pubertal age of onset of this tumour (4, 5).

Treatment of osteosarcoma consists of pre-operative and postoperative chemotherapy in combination with surgical resection of the tumour. Limb salvage has largely replaced amputation for local tumour control. Several chemotherapeutic agents have been tested in clinical trials, in which the currently used combination of doxorubicin, cisplatin and methotrexate was found to be the most effective (8). Despite the harsh chemotherapeutic regime, about 45% of the patients develop distant metastases, most often in the lungs (85%) (8, 9). Since the introduction of the chemotherapy in the 1980s survival has reached a plateau of 65-70% (10, 11). Therefore, new treatment options for osteosarcoma are desperately needed.

Osteosarcoma can be divided in several subtypes (5, 12). The focus of this thesis is on conventional osteosarcoma. Conventional osteosarcoma can be further
subdivided, based on the predominant matrix, in the osteoblastic (76-80% of the cases), the chondroblastic (10-13%) and the fibroblastic (10%) variant (5, 13, 14). Less common subtypes of conventional osteosarcoma are the giant cell rich, osteoblastoma-like, epithelioid, clear cell and chondroblastoma-like subtype (5). Although the proportion of good histological responders, defined as >90% chemotherapy induced tumour necrosis, differs significantly between the conventional osteosarcoma subtypes, there is no difference in the overall survival (14).

**Osteosarcoma cell of origin**

The cell of origin of osteosarcoma is not well defined (15). However, two candidate progenitor cells are discussed in literature. The first hypothesis is that osteosarcoma arises from mesenchymal stem cells (MSCs). MSCs have the capability to differentiate into multiple lineages, such as myocytes, adipocytes, chondrocytes and osteocytes. These lineages of differentiation are respectively initiated by MyoD, PPARγ, SOX9, and osterix/Runx2 (16, 17). The capacity of MSCs to differentiate in multiple lineages resembles the histological spectrum observed in osteosarcoma (18). In addition, it has been suggested that the timing of the mutation can explain the different stages of differentiation observed in osteosarcoma, where an early genetic or epigenetic alteration may result in the development of an highly aggressive, undifferentiated osteosarcoma and vice versa (17, 19). Further evidence favouring the theory that osteosarcoma arises from MSCs comes from *in vitro* and *in vivo* studies, and from observations in the clinic. *In vitro*, our group and others have shown that mouse MSCs can undergo spontaneous transformation after long time culturing *in vitro* (18, 20-23) and that these MSCs formed osteosarcomas upon *in vivo* grafting (18). However, human MSCs do not show spontaneous *in vitro* transformation, even after prolonged culturing (24-27). In addition, publications reporting spontaneous human stem cell transformation have been retracted (28, 29) and can most likely be attributed to cross-contamination. Clinical observations also provide evidence favouring the mesenchymal stem cell theory, as osteosarcoma formation is found in patients following bone-marrow transplantation for unrelated diseases (30, 31). In addition, the existence of primary extraskeletal osteosarcoma supports this theory as in this rare subtype, osteosarcoma arises in tissues where no osteoblasts are present. The second theory hypothesizes
that osteosarcomas arise from osteoblast-committed cells. Evidence for this theory stems mostly from studies in mice that show that osteoblast specific knockout of p53 (and Rb) can induce murine osteosarcoma (32-36). More specifically, Walkley et al. and Berman et al. made an osterix-cre-mediated deletion of p53 and pRb, which resulted in osteosarcoma formation (32, 35). As mentioned above, osterix is a transcription factor that is required for osteoblast differentiation and bone formation (37). Interestingly, p53 (and Rb) knockdown in undifferentiated mesenchymal cells also results in the formation of several sarcomas, among which osteosarcoma is the most frequent one (33). One study by Quist et al. compared osteosarcoma formation in Prx1-Cre, Collagen-1α1-Cre and Osteocalcin-Cre conditional disruption of p53 and Rb to transform undifferentiated mesenchyme, preosteoblasts and mature osteoblast, respectively (36). The Prx1 and Collagen-1α1 had a near complete penetrance of osteosarcoma, whereas only 44% of the osteocalcin-cre mice mice developed osteosarcomas (36). Although there seems to be increasing evidence supporting the first hypothesis, the differentiation state of the progenitor cells remain a point of debate among those who study this disease.

The IGF pathway

The IGF pathway has two closely related ligands: IGF1 and IGF2. IGF2 is an imprinted gene, only expressed from the paternal allele; IGF1 is mainly produced by the liver upon stimulation by the growth hormone (GH). The GH is produced by the pituitary gland and under control of the hypothalamus, as the hypothalamus produces the growth hormone releasing hormone (GHRH) and the growth hormone-inhibiting hormone (GHIH) (also known as somatostatin) which balance determines the amount of GH that is released. Besides the function of IGF1 and IGF2 as endocrine hormones, they can also act in autocrine and paracrine manners (38). In the circulation, six IGF binding proteins (IGFBPs) can bind IGF1 and IGF2 (39). IGFBPs are expressed in many peripheral tissues, except for IGFBP1 which is mainly produced by the liver (40). These binding proteins increase the half-lives of the IGF ligands, but they also interfere in the interaction between the IGF ligands and their receptors. IGFBP3 is the most abundant protein, which forms a ternary complex with insulin-like growth factor acid-labile subunit (IGFALS) and accounts for 80% of all IGF binding.

IGF1 and IGF2 can bind to the IGF1 receptor (IGF1R). Unlike IGF1, IGF2
can additionally bind to the insulin receptor (IR) with high affinity (Figure 1). Two monomeric isomers of the IR have been identified: IRA (lacking exon 11) and IRB (with exon 11). These alternative splice variants differ in the C-terminus, where they influence receptor-ligand interaction (41). IGF2, in addition to binding to the IR and the IGF1R, can bind the IGF2 receptor (IGF2R). The IGF2R does not confer intracellular signalling but functions as a decoy receptor to decrease the availability of IGF2 to the IGF1R (42). Upon ligand binding, the IGF1R can form homodimers or hybrid receptors with the IR. The α chains of the IGF1R (which are located extracellular) induce tyrosine autophosphorylation of the β chains (which span the membrane) (43). This phosphorylation recruits the downstream signalling protein insulin receptor substrate (IRS) to the cell membrane (43). Phosphorylation of IRS creates binding sites for SH2 domain of various proteins, which finally leads to the activation of the PI3K/AKT/mTOR pathway and the Ras/Raf/ERK signalling pathways (43). Both these pathways are involved in many cellular functions, such as cell growth, proliferation and differentiation (44).

**Indications for IGF pathway involvement in osteosarcoma genesis**

In normal development, IGF1R signalling plays a key role in the growth and development of bone. This is illustrated in mice, as IGF1R knockout mice are approximately 55% smaller compared to IGF1R wildtype mice (45). Interestingly, in dogs, the size of different breeds is dependent on IGF1 plasma levels (46). Due to the role of the IGF pathway in normal bone development, the IGF pathway has been studied for decades in osteosarcoma. Interestingly, the peak incidence of osteosarcoma correlates with the increased levels of GH and IGF ligands in puberty (Figure 2). Furthermore, two studies suggest that height at diagnosis is a risk factor of osteosarcoma (47, 48). In addition, a few cases of osteosarcoma in patients with acromegaly are reported (49) and patients with a congenital IGF1 deficiency seem protected against cancer (50). Multiple GH treated patients presented with osteosarcoma (51, 52).

Strikingly, a recent study identified mutations in IGF signalling genes in 7% and IGF1R amplification in 14% of osteosarcoma cases (53). These clinical observations combined with the role of the IGF pathway in normal bone development strongly suggest a role for the IGF pathway in osteosarcoma genesis.
Figure 1. The components of the IGF pathway, adapted from van Maldegem et al. (54).
General introduction and outline of this thesis

Chondrosarcoma is the second most common type of malignant bone tumour. It accounts for 20% of all bone cancers and is characterized by the production of cartilage. It is most common in the 3rd to 6th decade of life (Figure 2), and most frequently occurs in the bones of the pelvis, the ribs, and bones of the extremities. In rare cases, they occur in the small bones of the hands and feet, or in the skull (56). Conventional chondrosarcoma is the most common subtype that accounts for 85% of the cases (57). This subtype can be histologically subdivided into atypical cartilaginous tumour (ACT), grade II and grade III chondrosarcomas (58). The ACT, previously known as grade I, accounts for 61% of the cases. The first line treatment is curettage with local adjuvant treatment, resulting in a 5-year survival rate of 95%. Grade II (36%) and grade III (3%) chondrosarcomas have a worse 5-year survival of 86% and 58%, respectively, due to the occurrence of metastases (59-61). These tumours are treated by en bloc resection with wide resection margins to prevent local recurrence. Chondrosarcoma patients with unresectable disease, due to tumour location, tumour size or extensive metastatic disease, have a 5 year survival of only 2% (62, 63). These chondrosarcoma patients slightly benefit from doxorubicin-based chemotherapy, which increases the 3 year survival from 8% to 26% (62).

Chondrosarcoma either arises in the metaphysis or epiphysis of the bones, in which case it is called a central chondrosarcoma, or it arises on the surface

Figure 2. The peak in IGF1 levels correlates to the peak incidence in osteosarcoma. Osteosarcoma incidence rates, chondrosarcoma incidence rates and IGF1 levels were derived from Mirabello et al. (55), WHO Classification of bone tumours 2013 and the Mayo Clinic, respectively.

Chondrosarcoma
of the bone, in which case it is called a peripheral chondrosarcoma. Central and peripheral differ in aetiology, demonstrated by their different genetic background (Figure 3).

**Central chondrosarcomas**

In addition to primary chondrosarcoma, where tumours arise without a precursor lesion, secondary chondrosarcomas can also arise, in which the tumour arises from a benign precursor lesion. In case of a central chondrosarcoma, this benign precursor lesion is the enchondroma (Figure 3). Enchondromas show a predilection for the small bones of the hands and feet, and occur over a wide age distribution (64). Ollier disease and Maffucci syndrome, where patients present with multiple enchondromas, suggest the presence of an underlying genetic alteration. Indeed, gain of function mutations in *isocitrate dehydrogenase 1 and -2 (IDH1 and -2)* have been identified as driver mutations of enchondromas (65-67). 52%- 87% of the enchondromas harbour an *IDH1/2* mutation (65, 66).

![Diagram of chondrosarcoma genetics](image)

**Figure 3.** Chondrosarcoma genetics. *EXT* mutations have been identified as potential driver mutations of osteochondromas, the benign precursor lesions of peripheral chondrosarcomas. *IDH1/2* mutations can give rise to enchondromas, which can then develop into central chondrosarcomas. However, central chondrosarcomas can also occur without the presence of a benign precursor lesion. Mutations in *p53* and *retinoblastoma (Rb)* and *CDKN2A* silencing have been identified in the development of low-grade to high-grade lesions.
IDH1 and IDH2 are essential enzymes in cell metabolism, as they convert isocitrate to α-ketoglutarate (α-KG) in respectively the cytoplasm and the mitochondria (Figure 4). The mutant enzyme acquires the activity to convert α-KG to D-2-hydroxyglutarate (D-2-HG), an oncometabolite that competitively inhibits the α-KG dependent enzymes by the high structural similarities (68). More specifically, D-2-HG inhibits histone demethylases and tet methylcytosine dioxygenase 2 (TET2)(68). TET2 is essential
for the conversion of the modified genomic base 5-methylcytosine to 5-hydroxymethylcytosine, the first step in cytosine demethylation. An aberrant methylation phenotype is observed in IDH1/2 mutated gliomas (69-72), leukaemia’s (73) and enchondromas (66).

**Peripheral chondrosarcomas**

Peripheral chondrosarcomas always arise secondary from osteochondromas. Osteochondromas are benign lesions, characterized by bony projections with a mature hyaline cartilaginous cap. They are most commonly located adjacent to the growth plate of the long bones (Figure 3) (74). Malignant transformation from a solitary osteochondroma to a secondary peripheral chondrosarcoma only occurs in 1% of the cases. Multiple osteochondromas are found in approximately 15% of the patients (75). When this is the case a patient has the hereditary autosomal dominant syndrome ‘Multiple Osteochondromas’ (MO), which is caused by a germline mutation in the *exostosin-1 (EXT1)* or – 2 (*EXT2*) gene (74-76). In the 10-15% of the multiple osteochondroma patients where no point mutation in *EXT1* or *EXT2* can be identified, somatic mosaicism with large genomic deletions is likely the underlying mechanism for osteochondroma genesis (77, 78). Somatic homozygous deletions of *EXT1* can also be found in sporadic osteochondromas (74, 75). Strikingly, secondary peripheral chondrosarcomas no longer contain the *EXT* mutations, suggesting that these arise from the wildtype *EXT* cells present in the lesion (79).

*EXT1* and *EXT2* are essential for heparan sulfate synthesis as they catalyse the elongation of the heparan sulfate chains. Heparan sulfate is a proteoglycan that regulates Indian Hedgehog (IHH) signalling in the growth plate (80, 81). IHH acts together with the parathyroid hormone-related protein in a negative feedback mechanism that regulates the balance between chondrocyte proliferation and differentiation in the growth plate (81-83). When IHH signalling is impaired due to a mutation in *EXT1* or *EXT2*, this results in less chondrocyte differentiation and increased chondrocyte proliferation, thereby causing osteochondroma genesis.

**Rare chondrosarcoma subtypes**

Dedifferentiated chondrosarcoma accounts for 10% of chondrosarcoma
patients (84). It is a highly malignant variant, reflected by the 28% 10-year survival that is seen in patients diagnosed without metastasis (85). Patients that are diagnosed with metastasis have an even worse prognosis, as only 10% survives the first two years (85). Dedifferentiated chondrosarcoma has a typical histology, as two clearly distinct compartments can be observed. The first is a well-differentiated cartilage tumour, usually an enchondroma or a low-grade conventional chondrosarcoma. This component is juxtaposed to a dedifferentiated, high-grade non-cartilaginous sarcoma (86). The dedifferentiated component determines patient prognosis, and is therefore the focus of research that aims to identify new therapeutic strategies.

In addition to genetic alterations that are present in many cancer types, such as \textit{p53} mutation, \textit{PTEN} deletion, \textit{MYC} amplification and \textit{MDM2} amplification (86-88), mutations in \textit{IDH1/2} have been identified in 54% of the dedifferentiated chondrosarcomas (86).

Mesenchymal chondrosarcoma is a rare aggressive subtype in which distant metastasis can be identified even after 20 years (89-91). It accounts for 3% of all chondrosarcoma cases, and has a 10-year survival of 43% (92). Small round undifferentiated cells with islands of differentiated cartilage histologically characterize this subtype. In addition to its occurrence in the skeleton (mainly in the craniofacial bones, the ribs and the vertebrae), 20-30% of the cases present with primary soft tissue localization (64, 89). Tumour localization in the jaw is a bad prognostic factor, as well as presentation with metastases, while a young age is potentially a good prognostic factor (93, 94).

In contrast to dedifferentiated chondrosarcomas, no mutations in \textit{IDH1/2} have been identified. Furthermore, mutations in \textit{p53}, deletions in \textit{p16} and \textit{MDM2} amplifications are rare events (86, 95, 96). Interestingly, mesenchymal chondrosarcomas carry the HEY1-NCOA2 fusion gene (97). Furthermore, in a case where this fusion was absent, an IRF2BP2-CDX1 fusion gene has been identified (98). This demonstrates that mesenchymal chondrosarcoma has a distinct genetic background.

Clear cell chondrosarcoma is a low-grade variant of chondrosarcoma, with a mortality of ~15% (99). It is the most rare subtype, as it accounts for only 2% of
all chondrosarcoma cases. While metastases are rare, clear cell chondrosarcomas recur locally after curettage in 86% of the cases (100, 101). Interestingly it has a male: female ratio of 2.6:1, and is therefore the only subtype that has a sex preference (100). It derived its name by the histological presentation, showing clear cells that are surrounded by hyaline cartilage (99). It is most commonly located at the epiphyseal ends of long bones, and in line with conventional chondrosarcoma, the best treatment option is en bloc resection with clear margins (99, 100). Clear cell chondrosarcomas have widespread chromosome gains and losses, often with hemizygous involvement of the \( p16 \) locus. No alterations in \( IDH1/2, p53 \) and \( MDM2 \) have been reported (86).

**Chondrosarcoma cell of origin**

Originally, it was believed that chondrosarcoma originates from remnants of the growth plate cartilage. Although the chondrosarcoma cell of origin remains uncertain, it is now widely supported that chondrosarcomas most likely originate from MSCs undergoing chondrogenic differentiation (102). The formation of chondroid callus during bone facture healing demonstrates the lifetime presence of these cells in bones. Furthermore, the existence of extraskeletal chondrogenic neoplasms suggests that these cells are even present outside the skeleton (103). A strong argument favouring the MSC as the cell of origin for central chondrosarcoma is provided by a study from our group, in which elevated levels of \( D-2-HG \) (the oncometabolite induced by an \( IDH1/2 \) mutation) blocked osteogenic differentiation and variably promoted chondrogenic differentiation of MSCs (104). For secondary peripheral chondrosarcoma, compelling evidence comes from studies in zebrafish and mice. Chondrocytes in \( Ext2 \)-null zebrafish have been shown not to undergo terminal differentiation, and pre-osteoblasts failed to differentiate towards osteoblasts (105). In mice, \( Ext1 \) inactivation in chondrocytes resulted in osteochondromas formation, the precursor lesion of secondary peripheral chondrosarcomas, thereby demonstrating that osteochondromas originate from proliferating chondroctyes in the growth plate cartilage (106, 107).

There are striking parallels between normal cartilage growth and differentiation and cartilage tumours (108). Histologically, distinct chondrosarcoma subtypes show high similarities with different zones of chondrocyte differentiation in the
normal growth plate (106). In addition, an analyses that evaluated expression levels of chondrogenesis-relevent genes demonstrated that grade I tumours cluster with chondrocytes, while grade III tumours cluster with earlier stages of MSCs (107). Due to these parallels, increasing our understanding of normal cartilage development will increase our understanding of chondrosarcoma genesis.

**Targeting chondrosarcoma cell metabolism**

Already in 1924, Otto Heinrich Warburg observed that cancer cells metabolize glucose in a different way than normal differentiated cells: while normal cells use mitochondrial oxidative phosphorylation to produce their energy, tumour cells mainly produce their energy via glucose fermentation, even in the presence of sufficient oxygen (110, 111). This phenomenon, later called “the Warburg effect”, was the first indication that cancer cell metabolism differs from normal cell metabolism. The aerobic glycolysis used by cancer cells is an inefficient way to generate adenosine 5’-triphosphate (ATP), but might be required to allow the production of nucleotides, amino acids and lipids, which are essential for cell proliferation (112). Despite the fact that the Warburg effect was identified almost a decade ago, relatively little progress had been made in increasing the understanding of how altered cell metabolism contributes to carcinogenesis. However, the recent identification of mutations in TCA cycle enzymes in several cancers, among which mutations in \textit{IDH1} and \textit{IDH2}, renewed the interests in cancer cell metabolism as this demonstrates that enzymes involved in cell metabolism play an important role in tumourigenesis. In 2011, Hanahan and Weinberg recognized the reprogramming of energy metabolism as a hallmark of cancer (113). The identification of mutations in TCA cycle enzymes opened new therapeutic possibilities.

When AGI-5198 was identified as a specific IDH1 mutant inhibitor (selective for the R132H mutation) (114), the field of chondrosarcoma immediately recognized its potential as chondrosarcoma therapy. However, our group has shown that treatment with AGI-5198 does not influence the tumorigenic properties of chondrosarcoma cells (115). In \textit{IDH2} mutant leukaemia, an initial proliferation burst followed by cellular differentiation was observed upon inhibition of the IDH2 mutant enzyme (116, 117). Furthermore, in \textit{IDH1} mutant glioma, the initially described impaired cell proliferation upon IDH1 mutant inhibition (114) could not be confirmed in other studies (118, 119).
In contrast to gliomas (120-122), the *IDH1/2* mutation status does not correlate with chondrosarcoma survival, and does not cause loss of 5-hydroxymethylcytosine or altered histone modifications in central chondrosarcomas (123). This suggests that while the *IDH1* or -2 mutations are an early event in tumorigenesis, at later stages other processes involved in chondrosarcoma progression render these cells independent of the mutant IDH1/2 enzymes. Therefore, we propose to target the altered metabolism caused by the *IDH1/2* mutations as therapeutic strategy for chondrosarcoma.

One pathway that is linked to cell metabolism and has been suggested to play a role in chondrosarcoma progression is the IGF pathway (124). IGFBP3, the binding protein that accounts for 80% of the IGF1 and IGF2 binding, is lower expressed in enchondromas than in normal growth plate cartilage, and expression levels decrease with increasing grades of chondrosarcoma (125). Furthermore, it has been described that IGF1 induces migration of chondrosarcoma cell lines, which can be blocked by an IGF1R antibody (126). Together with collaborators from the Ludwig Center at Dana-Faber/Harvard, our group performed functional profiling of Receptor Tyrosine Kinases in chondrosarcomas (127). IGF1Rβ and IRβ hypermethylation was identified in 1 out of 5 cell lines tested; an IGF1R/IR inhibitor could inhibit proliferation of this cell line (127). Taken together, these studies suggest a potential role for the IGF pathway in chondrosarcoma development, migration and proliferation.

It has been described that the *IDH1/2* mutation influences nicotinamide adenine dinucleotide (NAD⁺) synthesis. More specifically, metabolic profiling of glioblastoma cells upon mutant IDH1 inhibition revealed nicotinamide phosphoribosyltransferase (NAMPT) as a therapeutic target for *IDH1/2* mutated tumours (128). An increased sensitivity for NAMPT inhibitors correlated with a decreased expression of nicotinic acid phosphoribosyltransferase (NAPRT) caused by an increased methylation of the NAPRT promoter (128). NAMPT and NAPRT are rate-limiting enzymes of the primary salvage pathway and the Preiss-handler pathway, respectively, which are involved in NAD⁺ synthesis. Tumour cells depend on these pathways for their rapid NAD turnover, as they lack expression of key enzymes in the de novo synthesis of NAD⁺ from tryptophan (129-131).
Another main metabolic pathway that has been identified as being altered by the IDH1/2 mutations is glutaminolysis (132-137), an important energy-producing pathway in tumour cells. Glutamine, a non-essential amino acid, can subsequently be converted to glutamate and α-ketoglutarate by glutaminase and glutamate dehydrogenase, respectively, thereby fuelling the TCA cycle and the D-2-HG production in IDH1/2 mutated cells. IDH1/2 mutated cells are more sensitive to several compounds that interfere with glutaminolysis compared to IDH1/2 wildtype cells, such as CB-839, metformin and phenformin. CB-839 is an inhibitor of glutaminase that decreases D-2-HG levels and inhibits growth of IDH1/2 mutated acute myeloid leukaemia cells (138). An endogenous heterozygous knock-in of the R132H IDH1 mutation sensitizes breast epithelial cells to metformin, the first in line antidiabetic drug, and its lipophilic analogue phenformin (133). Therefore, inhibition of NAD⁺ synthesis or glutaminolysis potentially leads to synthetic lethality with the mutated IDH1/2 enzyme in chondrosarcoma.

**Aim and Outline of the thesis**

The aim of this thesis was to develop new therapeutic strategies by identifying cellular pathways that are essential for chondrosarcoma and osteosarcoma cell survival.

Chapters 2, 3 and 4 are focussed on osteosarcoma. Although osteosarcoma is a rare disease, many preclinical experiments with osteosarcoma cells have been published. In Chapter 2, a systemic analysis of the literature on osteosarcoma in vitro studies is given to determine how the quantity of these studies developed over time. The downside of this huge number of studies is discussed from a researcher’s perspective. Chapter 3 reports on the analyses of genome-wide gene expression data, where overexpression of the IGF pathway in osteosarcoma is identified compared to osteoblasts and MSCs. Osteosarcoma cell lines were treated with an IGF1R/IR dual inhibitor.¹ In Chapter 4, a commentary on a similar transcriptional profiling study is given. An overview of where we are with IGF1R-directed therapy, where we need to go and how we get there is presented.

¹ The gene set analysis described in Chapter 3 was performed by dr. M.L. Kuijjer. E.F.P. Peterse performed the Western blotting and the Proliferation assays.
Chapters 5, 6 and 7 are focussed on chondrosarcoma. In Chapter 5, the potency of targeting the IGF pathway in chondrosarcoma is explored, as previous studies suggested the IGF pathway as a potential therapeutic target. IGF1R expression levels are evaluated in cartilage tumours, and expression of IGF1R signalling mediators and sensitivity for IGF1R inhibition is evaluated in chondrosarcoma cell lines. In chapters 6 and 7, metabolic pathways altered by the \( IDH1/2 \) mutation are explored as therapeutic strategy. In Chapter 6, interference in NAD\(^+\) synthesis pathway is evaluated, as vulnerability for NAD\(^+\) depletion is reported for \( IDH1/2 \) mutant cells. Chondrosarcoma cell lines were treated with NAMPT inhibitors, and NAPRT expression and methylation was evaluated in cartilage tumours using qRT-PCR and genome-wide methylation arrays. Chapter 7 describes a preclinical study evaluating the effect of interfering in chondrosarcoma cell glutaminolysis. The expression of glutaminase, the first essential enzyme of glutaminolysis, is compared between low-grade and high-grade chondrosarcomas to see if this pathway is involved in chondrosarcoma progression. In addition, chondrosarcoma cell lines are treated with the glutaminase inhibitor CB-839, and the compounds metformin, phenformin and chloroquine that all inhibit glutaminolysis at different levels. In Chapter 8, results described in chapters 2 to 7 are discussed and future perspectives are given.
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