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

Jong, Y. de

Chapter 1

General introduction and Thesis outline

Chondrosarcoma

Chondrosarcoma is a malignant cartilage tumour representing 20% of malignant bone tumours [1]. It is most common in adults around the age of fifty and develops predominantly in the bones of the ribs, pelvis and bones of the extremities. The most frequently observed subtype is conventional chondrosarcoma, which represents 85% of all the chondrosarcomas, followed by rarer chondrosarcoma subtypes dedifferentiated chondrosarcoma (10%) [2], mesenchymal chondrosarcoma (2%) [3], clear cell chondrosarcoma (2%) [4] and periosteal chondrosarcoma (1%) [1] (see figure 1).

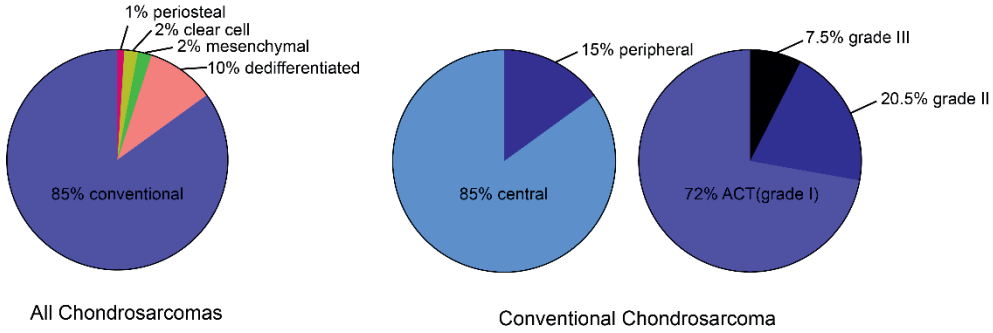

Figure 1. Chondrosarcoma subtypes and grades. Conventional chondrosarcomas are the most common subtype, followed by dedifferentiated chondrosarcomas. Of all conventional chondrosarcomas, central chondrosarcoma is most frequently observed.

Conventional Chondrosarcoma

Conventional chondrosarcoma can be found either as a central subtype, when it is located inside the medulla of the bone or as a peripheral subtype when it is located next to the bone. Central conventional chondrosarcoma accounts for 85% of all conventional chondrosarcomas, while peripheral chondrosarcoma is rarer and comprises 15% of all conventional chondrosarcomas. Conventional chondrosarcoma can be further subdivided into three histologically different grades; Atypical cartilaginous tumour /Grade I) (72%), Grade II (20.5%) and Grade III (7.5%) [5], which histologically show an increase in cellularity and a decrease in matrix deposition with increasing grade (see figure 2). Based on the latest WHO classification from 2020 the term Atypical cartilaginous tumour is given to

low grade cartilaginous lesions located in the long and short tubular bones, while the term Grade I is restricted for low grade cartilaginous lesions located in the flat bones. The histological appearance is similar, but lesions located at the flat bones represent with a poorer clinical outcome, and need more extensive surgery [6]. Histological grade is the most important predictor of survival and metastasis. Atypical cartilage tumours rarely metastasize and show a relatively good prognosis with a 10-year survival rate of 83-88%. Grade II chondrosarcomas show a 10 years survival of 62-64% and grade III chondrosarcomas show a very poor prognosis with a 10 years survival rate of 26-29% and a high risk of developing metastasis [1, 5, 7, 8]. Recurrence of a low-grade tumour into a higher histological grade occurs in ~13% of the cases, leading to a worse prognosis for these patients [7, 9].

Central chondrosarcomas can arise *de novo* or from a pre-existing enchondroma, which are benign cartilage lesions that can arise in the medulla of the bone. Characteristic mutations identified in 87% of enchondromas and 38-70% of central chondrosarcomas are mutations in *isocitrate dehydrogenase 1 (IDH1)* or *isocitrate dehydrogenase 2 (IDH2)* [10, 11]. These are enzymes catalysing the conversion of isocitrate to alpha-ketoglutarate in the Krebs Cycle. A mutation in one of these genes leads to a gain of function in which alpha-ketoglutarate is converted to the oncometabolite D2HG (See figure 3). This oncometabolite competes with alpha-ketoglutarate to bind to alpha ketoglutarate dependent enzymes resulting in inhibition of these enzymes. This group of enzymes is involved in different important processes such as methylation, collagen folding and oxygen sensing [12-14].

Peripheral chondrosarcomas always develop from a pre-existing benign osteochondroma. Osteochondromas are benign bone tumours developing adjacent to the bone, which can progress towards secondary peripheral ACT/CS1. Osteochondromas can either occur as sporadic or as hereditary disease in which case multiple osteochondromas develop. Alterations in *Exostosin glycosyltransferase 1 (EXT1)* or *Exostosin glycosyltransferase 2 (EXT2)* are involved in the development of osteochondromas. Heterozygous germline mutations in these genes have been identified in 70-95% of patients with multiple osteochondromas [15]. In addition, homozygous inactivation of *EXT1* is observed in 80% of sporadic osteochondromas [16-18]. Although homozygous inactivation is observed in osteochondromas, only a small percentage (~15%) of peripheral chondrosarcomas show homozygous inactivation of the *EXT* genes [18-22]. This leads to the

hypothesis that wildtype cells with functional EXT that are located in or near this EXT-null microenvironment are the precursors of secondary peripheral chondrosarcoma [23]. EXT1 and EXT2 are involved in biosynthesis of heparan sulfate chains of proteoglycans [24]. Proteoglycans are important for cell to cell contact and regulate growth plate organization. The absence of EXT1 or EXT2 in the developing bone will lead to the formation of an osteochondroma [25].

Rare chondrosarcoma subtypes

Dedifferentiated chondrosarcoma is a malignant high-grade chondrosarcoma subtype. Histologically it shows a mixed appearance of a usually low grade cartilaginous component and a high grade dedifferentiated component [2] (see figure 2). The 10 years survival is only 28%, and when the patient presents with metastasis at diagnosis this if further decreased to 10% [26]. Like in conventional central chondrosarcomas *IDH1* and *IDH2* mutations have been identified in 54% of the cases [10].

Mesenchymal chondrosarcoma is a high-grade tumour with a reported ten years survival rate between 27 and 67% [27, 28]. Its histology shows a mixture of differentiated cartilage combined with undifferentiated small round cells [3] (see figure 2). Genetically, mesenchymal chondrosarcoma is characterized by a fusion between *HEY1* (Hes-Related Family BHLH Transcription Factor with YRPW Motif 1) and *NCOA2* (Nuclear Receptor Coactivator 2). The function of HEY1 is as transcription factor downstream of Notch signalling. NCOA2 is a nuclear hormone receptor coactivator. Their role in development of mesenchymal chondrosarcoma still needs to be investigated.

Clear cell chondrosarcoma is a low-grade chondrosarcoma subtype histologically showing malignant cells with clear empty cytoplasm and hyaline cartilage matrix (see figure 2). It is mainly found in the epiphysis of the femoral or humoral head and the prognosis is relatively good with a mortality rate of approximately 15% [29]. No recurrent possible initiating mutations have been identified in this subtype.

Periosteal Chondrosarcoma originates from the periosteum and is a very rare chondrosarcoma subtype accounting for less than 1% of the cases [1]. Little is known about its development and progression however *IDH1* mutations have been identified in 15% of periosteal chondrosarcomas [30].

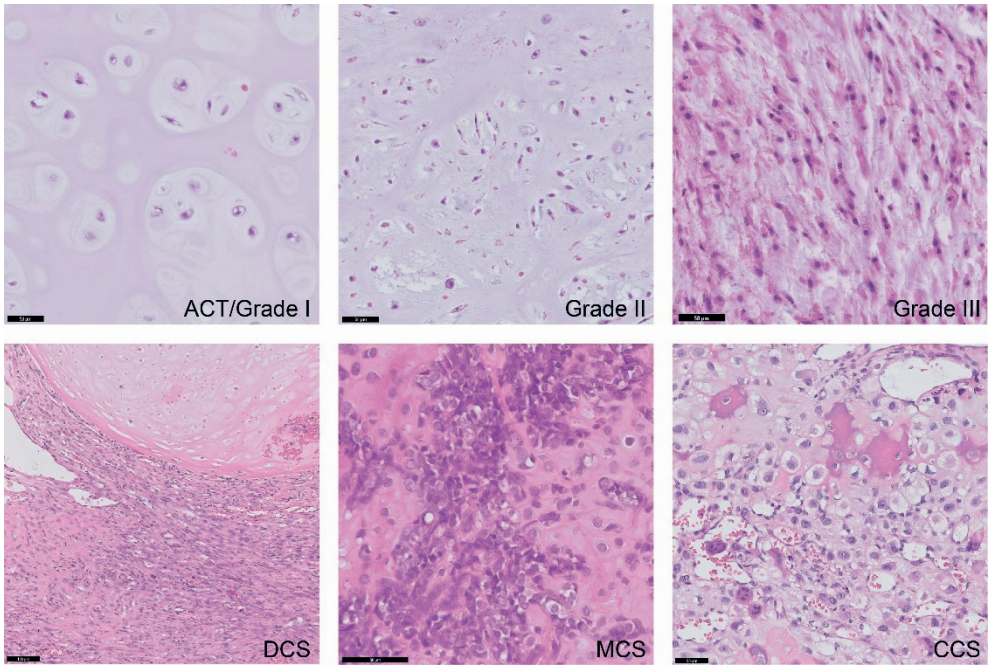

Figure 2. Histological appearance of conventional (ACT, Grade II and Grade III) and rare chondrosarcoma subtypes. (ACT: atypical cartilage tumour, DCS: dedifferentiated chondrosarcoma, MCS: mesenchymal chondrosarcoma, CCS: clear cell chondrosarcoma).

Current treatment options for patients with chondrosarcoma

Surgery

Chondrosarcomas are relatively resistant towards chemo- and radiotherapy, consequently the best treatment option for patients with chondrosarcoma is surgery. Complete surgical removal of the tumour provides the only chance for cure. Grade II and III chondrosarcoma is treated if possible, by wide, *en-bloc* excision of the tumour, while atypical cartilaginous tumour that is confined to the tubular bone, is removed by intralesional curettage often followed by adjuvant treatment. When the tumour is large or has grown into the surrounding soft tissue wide resection is always performed [8].

Radiotherapy

Chondrosarcomas are considered radioresistant, however after incomplete resection or when surgery is not feasible radiotherapy is given to

chondrosarcoma patients. This is challenging since high doses are needed to achieve an improvement especially when the tumour is located in the skull or spine [8]. Proton beam therapy has shown to be a much better option compared to conventional radiotherapy [31], especially for tumours close to vital organs. This type of radiotherapy can deliver the particles precisely to the tumour without damaging the surrounding tissue and since last year two centres in the Netherlands are able to offer treatment with proton radiotherapy.

Chemotherapy

Chemotherapy is not standard treatment for patients with chondrosarcoma, since there is no evidence whether this is beneficial for these patients. A small increase in median overall survival was observed in patients with unresectable chondrosarcoma when treated with conventional chemotherapy [32]. Mesenchymal chondrosarcoma has been described in several reports to be more sensitive to chemotherapy compared to other chondrosarcoma subtypes, and has shown a reduced risk of recurrence and a better overall survival after chemotherapy [27, 28, 33-38]. However in a meta-analysis this could not be confirmed [39].

Therapy resistance mechanisms

There are several possible explanations why chondrosarcoma cells are resistant to chemo- and radiotherapy; the tumour is slow growing and has a large amount of cartilage matrix, which may hamper access to the cells. This is however not the case for high grade chondrosarcomas, which proliferate faster and have myxoid instead of hyaline cartilaginous matrix, and they also show chemo- and radiotherapy resistance. A second possible mechanism is the expression of multi-drug resistance pumps, which has been shown on chondrosarcoma cell lines and patient tissues. However, despite the expression of these pumps doxorubicin was still able to accumulate in the nuclei of 3D chondrosarcoma cell pellets [40-42]. Another possible mechanism is the expression of anti-apoptotic Bcl-2 family members, which has been shown previously as a mechanism of chemoresistance in chondrosarcoma cell lines. Inhibition of Bcl-2 family members could sensitize conventional, dedifferentiated and, as shown in this thesis, mesenchymal chondrosarcoma cells towards doxorubicin and cisplatin chemotherapy [41, 43]. Furthermore, upregulation of other pro-

survival and down regulation of pro-cell death mechanisms are likely to play a role in therapy resistance.

Possible new therapeutic options

IDH1 and IDH2 mutations as therapeutic vulnerability

Mutations in *IDH1* or *IDH2* have been identified in 38-70% of central chondrosarcomas, 87% of enchondromas, 54% of dedifferentiated chondrosarcomas [10, 11] and 15% of periosteal chondrosarcomas [30]. Compounds specifically targeting mutant IDH1 or IDH2 have been developed by several pharmaceutical companies. Inhibiting mutant IDH1 using AGI-5198 decreased the production of D2HG in in vitro chondrosarcoma cell models, but did not lead to any reduction in viability, proliferation and migration, unless used at high, probably toxic dosages [44, 45]. This indicates that the mutation in *IDH1* or *IDH2* is an early event in tumorigenesis, but after progression chondrosarcomas are not dependent on it anymore. Although preclinical studies do not seem promising, clinical trials assessing the efficacy of IDH1 and IDH2 mutant inhibitors in chondrosarcoma patients are ongoing (NCT022737399, NCT02481154, NCT02073994, NCT03684811).

The oncometabolite D2HG competes with alpha-ketoglutarate to bind to alpha ketoglutarate dependent enzymes, which leads to inhibition of these enzymes [12-14] (Figure 3). Two groups of alpha ketoglutarate dependent enzymes that affect methylation are demethylating enzymes of the ten eleven translocation (TET) family and histone demethylases of the jumonji C domain containing (JMJD) family. TET enzymes convert 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC). Inhibition of TETs will lead to an increase in methylation. Indeed *IDH1* and *IDH2* mutant chondrosarcoma cell lines and tissues do show a CpG island methylator phenotype (CIMP) [11, 45, 46], however surprisingly no differences in 5-mC and 5 hmC immunostaining were observed in a panel of ~100 chondrosarcomas between *IDH1* or *IDH2* mutant and wildtype [47]. Likewise, no difference in histone methylation marks H3K27me3, H3K9me3 and H3K27me3 was observed in the same cohort. Further studies should determine whether *IDH1* or *IDH2* mutant chondrosarcoma patients should be included in clinical trials assessing the effect of demethylating compounds specifically in *IDH1* and *IDH2* mutant tumours (NCT03666559).

IDH1 and *IDH2* mutated cells produce very high concentrations of D2HG. For this process high amounts of alpha ketoglutarate are needed, which can be generated either by glycolysis or glutaminolysis. In *IDH1* and *IDH2* mutant cells generation of alpha ketoglutarate by glutaminolysis has been shown as the most important mechanism [48-50], making targeting glutamine metabolism an interesting vulnerability to specifically target *IDH1* or *IDH2* mutated cells. *In vitro* studies in chondrosarcoma cell lines show sensitivity towards glutamine pathway inhibitors, however in contrast to studies in genetically engineered cell lines [49, 51], no correlation to *IDH1* or *IDH2* mutation status was observed [52]. Clinical trials currently conducted in *IDH1* and *IDH2* mutated tumours, including chondrosarcoma, aim at inhibiting glutamine metabolism using either CB-839 (NCT02071862) or metformin or chloroquine (NCT02496741) [53].

The NAD+ synthesis pathway has been identified using metabolic profiling strategies as another *IDH1* and *IDH2* mutation specific vulnerability [54]. Although chondrosarcoma cells were extremely sensitive to inhibition of NAD+ synthesis, no correlation with mutation status could be identified [55]. Unfortunately phase I clinical trials including FK866 and GMX1778 (and prodrug GMX1777) were discontinued due to dose-limiting toxicities [56].

Another proposed vulnerability in *IDH1* and *IDH2* mutant cells is the increase in reactive oxygen species (ROS) levels due to a decrease in NADPH levels. NADPH is important for the reduction of ROS and in addition it is important for DNA damage repair. A decrease in NADPH, and consequently more ROS and less efficient DNA damage repair will increase sensitivity towards chemo and radiotherapy [57]. A better overall survival in *IDH1* and *IDH2* mutated cancers, most likely due to increased therapy sensitivity, has been reported in glioma [58] and glioblastoma [59], but not for acute myeloid leukaemia [60]. For chondrosarcoma the general consensus is that there is no change in outcome although literature studies show conflicting results [47, 61].

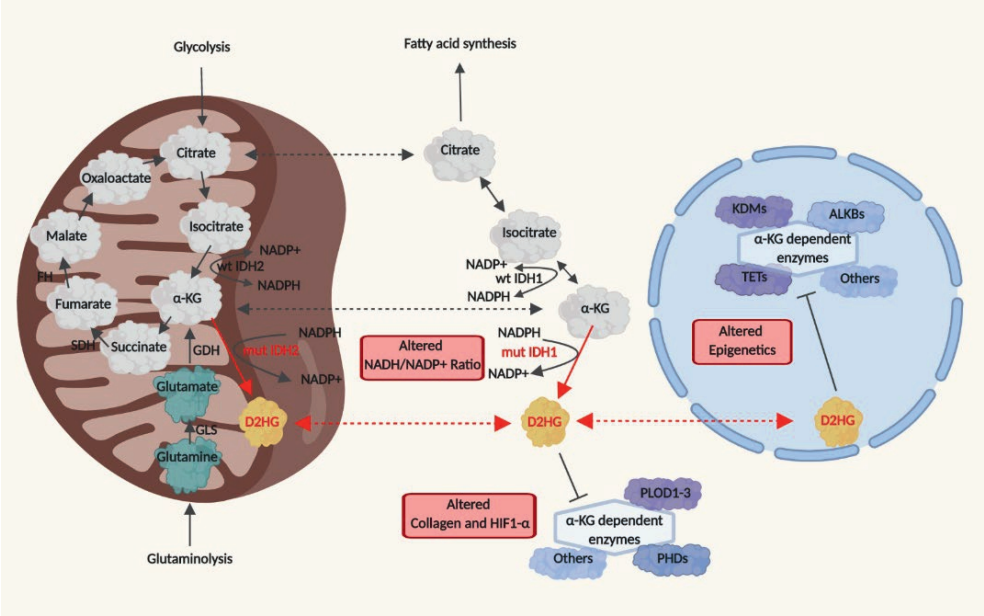

Figure 3. Citric acid cycle and (possible) consequences of *IDH1* or *IDH2* mutations on downstream cellular processes. Wild type IDH1 (cytoplasm) or IDH2 (mitochondria) converts isocitrate to α-ketoglutarate (α-KG), while mutant IDH1 or IDH2 converts α-KG to the oncometabolite D2HG. This process results in an altered NADH/NADP+ ratio. Furthermore the produced oncometabolite D2HG competes with α-KG to bind to α-KG dependent dioxygenases in the nucleus, resulting in altered epigenetics. In the cytoplasm inhibition of α-KG dependent dioxygenases results in alterations in collagen maturation and degradation of HIF1-α. In addition other not yet investigated enzymes are likely to be affected by the production of D2HG. Figure based on [52, 62].

Targeting anti-apoptosis proteins to induce chemosensitization

Apoptosis is a tightly regulated and controlled process of programmed cell death to remove damaged or unnecessary cells from the body. Apoptosis can be activated by either the intrinsic or the extrinsic pathway (figure 4). By upregulating anti-apoptotic proteins, cells can increase the threshold of DNA damage that can accumulate in a cell, and consequently cells can continue to divide in the presence of otherwise too much DNA damage [63]. As briefly mentioned above, chondrosarcoma cell lines express high levels of Bcl-2 and Bcl-xl, and upon inhibition with ABT-737 (inhibiting Bcl-2, Bcl-xl and Bcl-w) conventional and dedifferentiated chondrosarcoma cells could be sensitized towards doxorubicin and cisplatin [41].

Targeting death receptors is another strategy exploited in chondrosarcomas. It was shown that chondrosarcoma cell lines were not sensitive towards monotherapy with a death receptor inhibitor (TRAIL inhibitor), however they could be sensitized towards doxorubicin [64, 65]. Furthermore, clinical trials have been conducted investigating the use of death receptor inhibitors (PRO95780) in treatment of chondrosarcoma (NCT00543712), and although the phase I trial seemed promising, the phase II trial was terminated due to low efficacy. Future studies focussing on combination strategies will determine whether there is a role for TRAIL inhibitors in future chondrosarcoma treatment [66].

TP53 mutations have been observed in 20 percent of chondrosarcomas and are only found in the more malignant high-grade subtypes [67-69]. As can be observed in figure 3 P53 is a main component of the apoptosis pathway and its absence is thought to lead to deregulation of apoptosis activation, although P53 has a broad range of other functions as well [70]. Clinical trials with a mutant P53 inhibitor (APR-246) [71] are ongoing in *TP53* mutated cancers and first results look promising [72, 73] (NCT02098343, NCT03072043, NCT02999893).

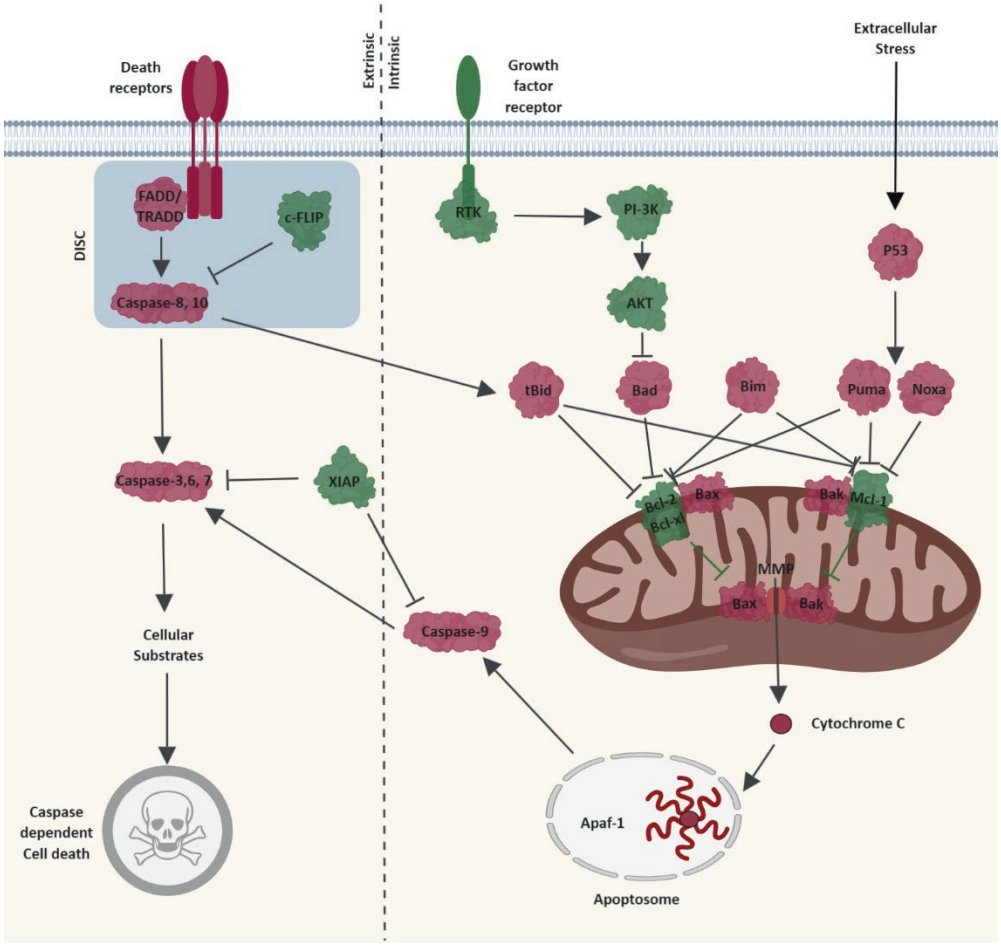

Figure 4. Apoptosis pathway. Schematic overview of the intrinsic and extrinsic apoptosis pathways. The intrinsic apoptosis pathway is activated upon extracellular stimuli, resulting in an upregulation of pro-apoptotic family members and a downregulation of anti-apoptotic family members. This eventually lead to mitochondrial membrane permeabilization (MMP) and the release of cytochrome C. This will bind to apaf-1 and the apoptosome is formed, this is able to cleave caspase-9 which in turn activates caspase 3, 6 and 7. The extrinsic apoptosis pathway is activated by death receptors and the formation of a death-inducing signalling complex (DISC) will result in activation of caspase 3, 6 and 7 either directly or through the activation of Bid, leading to MMP. Figure based on [74].

Upregulated pro-survival mechanisms as possible targets

Survival pathways in cancer cells are usually up or de-regulated and small molecules specifically inhibiting these pathways can serve as possible treatment options. In chondrosarcoma several pro-survival pathways have been shown to be upregulated (see figure 5). Kinome profiling of chondrosarcoma cell lines and primary cultures revealed that the SRC pathway was one of the most active pathways in chondrosarcoma [75]. Treatment of cell lines with dasatinib resulted in a dose dependent decrease in viability and sensitization towards doxorubicin especially in *TP53* mutant cells. In addition, SRC inhibition slowed down migration of chondrosarcoma cell lines.[76]. Treatment of low to intermediate grade chondrosarcoma with dasatinib resulted in stable disease for a small subset of patients indicating that treatment might be beneficial in some specific patient groups [77].

One growth factor receptor found to be active in chondrosarcoma is the platelet-derived growth factor receptor (PDGFR) and expression of PDGFR-alpha was correlated with adverse outcome [78, 79]. Although there was activation of the pathway, no increase in survival was observed in a phase 2 clinical study in which chondrosarcoma patients were treated with imatinib [80]. Regorafenib, a multi-kinase inhibitor, targeting VEGFR-1, 2, 3, TIE2, KIT, RET, RAF, PDGFR and FGFR is tested in a phase 2 study (NCT02389244) in metastatic bone sarcomas and first results in osteosarcoma look promising [81]. Expression of VEGF and presence of microvasculature has been shown in high grade chondrosarcoma, indicating that angiogenesis inhibition could be a potential therapeutic option for these patients [82-84]. A small case series including 10 patients treated with either pazopanib or ramucirumab suggests a potential benefit for patients with metastatic chondrosarcoma [85]. The current trial testing pazopanib in unresectable chondrosarcoma should prove whether inhibition of angiogenesis is a viable therapeutic strategy to treat high grade chondrosarcoma (NCT01330966).

Activating mutations in NRAS have been identified in 12% of chondrosarcoma samples indicating a role for MAP kinase inhibitors in the treatment of chondrosarcoma [86]. No clinical studies have been described so far including chondrosarcoma patients.

Another important pro-survival pathway upregulated in chondrosarcoma is the mTOR pathway. This pathway is active in 69% of conventional and 44% of dedifferentiated chondrosarcoma as shown by S6 phosphorylation [86]. Treatment of chondrosarcoma cell lines with a dual PI3K/mTORC1/2

inhibitor (dactolisib) resulted in a dose dependent decrease in viability. In addition a reduction in tumour growth was observed in an orthotopic mouse model after treatment [86]. Everolimus treatment in a rat chondrosarcoma model showed a block in cell proliferation and a decrease in Glut1 and HIF1a expression, two genes regulated by mTOR [87]. Combination treatment of rapamycin and cyclophosphamide showed a partial response in 1 and stable disease in 6 out of 10 patients. Confirmation of these results is currently ongoing in a phase II clinical trial (NCT02821507).

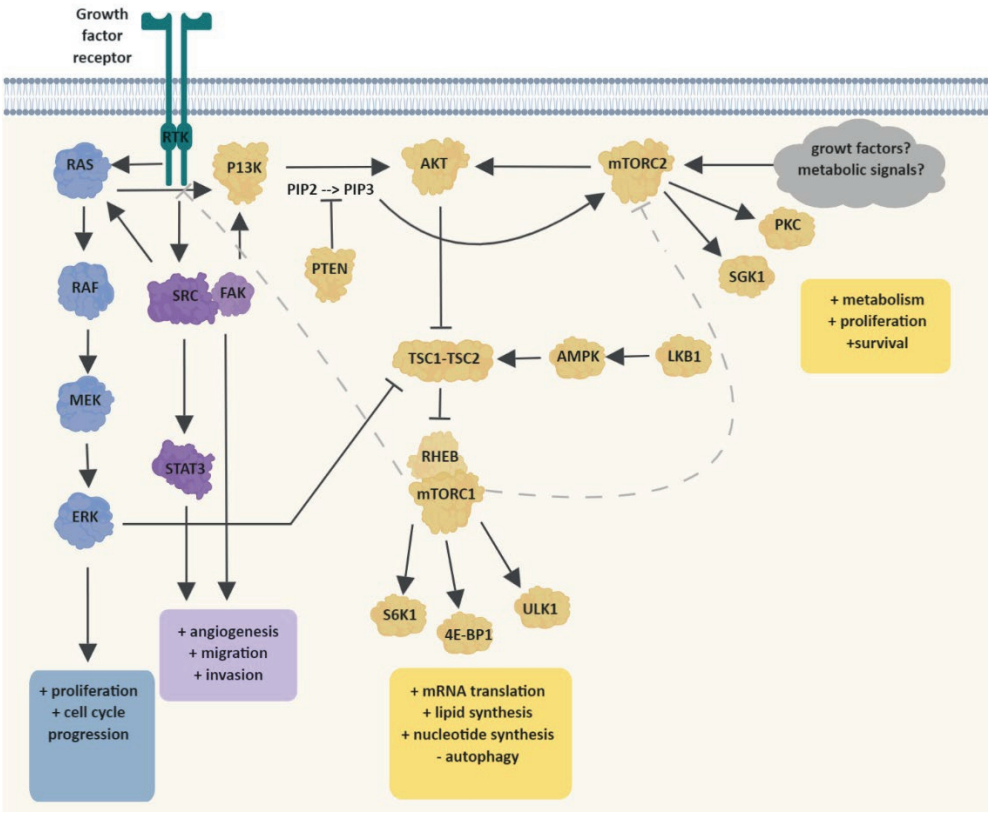

Figure 5. Survival pathways shown to be deregulated in chondrosarcoma. Simple representation of the mTOR pathway (yellow), RAS pathway (blue) and SRC pathway (purple) and their crosstalk. The mTOR pathway is activated by several growth factor receptor tyrosine kinases that can activate Pi3K. Phosphorylation of Phosphatidylinositol 4,5-biphosphate (PIP2) to generate Phosphatidylinositol 3,4,5-triphosphate (PIP3) activates AKT kinases, which in turn phosphorylate tuberous sclerosis protein 1 and 2 (TSC1-TSC2) leading to dissociation of the complex. This complex negatively regulates mTORC1, and therefore its dissociation leads to activation of mTORC1, which leads to an increase in mRNA translation, lipid and

nucleotide synthesis and a decrease in autophagy through the activation of amongst others; S6K1, 4E-BP1 and ULK1. The grey lines represent a negative feedback loop in which mTORC1 prevents the overactivation of the pathway. mTORC2 is less studied, but its activation is caused by PIP3 and growth factors as well as metabolic stimuli. mTORC2 activation leads to activation of amongst others AKT, PKC and SGK1 and will lead to activation of mTORC1, and an increase in metabolism and proliferation and survival. Activation of the Ras-Raf-Mek-Erk pathway is also induced by growth factor receptors and activation leads to an increase in proliferation and cell cycle progression. Ras can also activate Pi3K, and Erk has also the capacity to dissociate the TSC1-TSC2 complex leading to activation of mTORC1. SRC kinases show a lot of crosstalk and are regulating several important survival pathways. They can activate the RAS as well as mTOR pathways and thereby promoting proliferation and cell survival. Activation of SRC kinases also leads to angiogenesis, migration, invasion and metastasis trough activation of focal adhesion kinase (FAK) and transcriptional factors (for example STAT3). Figure based on [88-90].

Developmental pathways that play a role in chondrosarcoma development

During bone development several pathways regulate the differentiation of chondrocytes in the growth plate. One pathway particularly important in the Indian hedgehog (IHH) pathway. Together with parathyroid hormone-like hormone (PTHLH) a gradient is created in which immature chondrocytes are able to terminally differentiate. When IHH is constitutively active terminal differentiation cannot be achieved and this can result in the development of an enchondroma [91]. Mutations in *IHH* genes are identified in 18% of chondrosarcomas suggesting a role for this pathway in development and progression of chondrosarcoma [69]. A phase II clinical trial studying the effect of IHH inhibition (GDC-0449) showed only modest activity [92]. Although good results were obtained in a tumour xenograft model [93], treatment with another IHH pathway inhibitor IPI-926 did not show an improvement in progression free survival in chondrosarcoma patients [94].

Targeting the immune micro-environment

Targeting the immune micro-environment has become a big focus in cancer research and also in the sarcoma field more research has been investigating the applicability of immune therapy [95, 96]. In conventional chondrosarcoma immune infiltrate was found to correlate with prognosis; a higher amount of immune infiltrate correlated with a better prognosis [97]. Although immune infiltrate was detected in conventional chondrosarcoma, in another study immunohistochemical staining showed that there was no expression of the immune checkpoint ligand Programmed death ligand 1

(PDL-1) in conventional chondrosarcoma, but this was only present in 44% of dedifferentiated chondrosarcoma. In addition a high amount of immune suppressive immune infiltrate was found in the latter chondrosarcoma subtype [98]. In a retrospective case study including 2 patients with chondrosarcoma a promising response was observed for one of the patients with metastatic dedifferentiated chondrosarcoma; a partial response was maintained even after 26 cycles with PDL-1 inhibitor nivolumab [99]. A large phase 2 clinical trial assessing the safety and activity of PDL-1 inhibitor Pembrolizumab included five chondrosarcoma patients of which one showed an objective response [100]. In addition, a case report was published describing a patient with conventional chondrosarcoma showing a near complete response by nivolumab treatment [101]. Clinical trials currently ongoing are investigating the effect of combined mTOR and PD-L1 inhibition (NCT03190174) or combined CTLA-4 and PD-L1 inhibitors (NCT02982486) in sarcoma. Future studies should determine how many and which chondrosarcoma patients will benefit from PDL-1 inhibition.

Other immunogenic cancer specific markers identified in chondrosarcoma include MAGE-A3, NYESO-1/LAGE-1a and PRAME antigens that might be targetable using T-cells specific for these antigens [102-104].

Cell cycle regulators in high grade chondrosarcoma

One of the characteristics of cancer is uncontrolled proliferation, caused by a deregulation in the cell cycle and identifying and targeting these proteins can be a good strategy to treat cancer. The cell cycle is regulated by a large amount of proteins that are active during specific phases in the cell cycle (figure 6). Alterations in the retinoblastoma (RB) pathways have been identified in 96% of high grade chondrosarcomas, as shown by overexpression of CDK4, $p16^{INK4A}$ or cyclin D1 in high grade chondrosarcoma tissue samples [105, 106]. In addition higher expression of CDK4 was correlated with a worse overall survival [107]. *In vivo* studies in an orthotopic chondrosarcoma mouse model showed a good response towards CDK4/6 inhibitor palbociclib, which has been FDA approved for the treatment of breast cancer [107]. A clinical trial assessing the efficacy of palbociclib is conducted in sarcoma, although it is unknown if chondrosarcoma patients will be included in these studies (NCT03242382). As mentioned previously, *TP53* mutations are identified in 20% of chondrosarcomas [67-69]. Cells with a defective P53 protein depend more on the G2 checkpoint to halt cell proliferation in case of DNA damage. This gives opportunities to induce synthetic lethality by inhibiting proteins

regulating the G2 checkpoint and select patients with defective P53 function for treatment with these inhibitors.

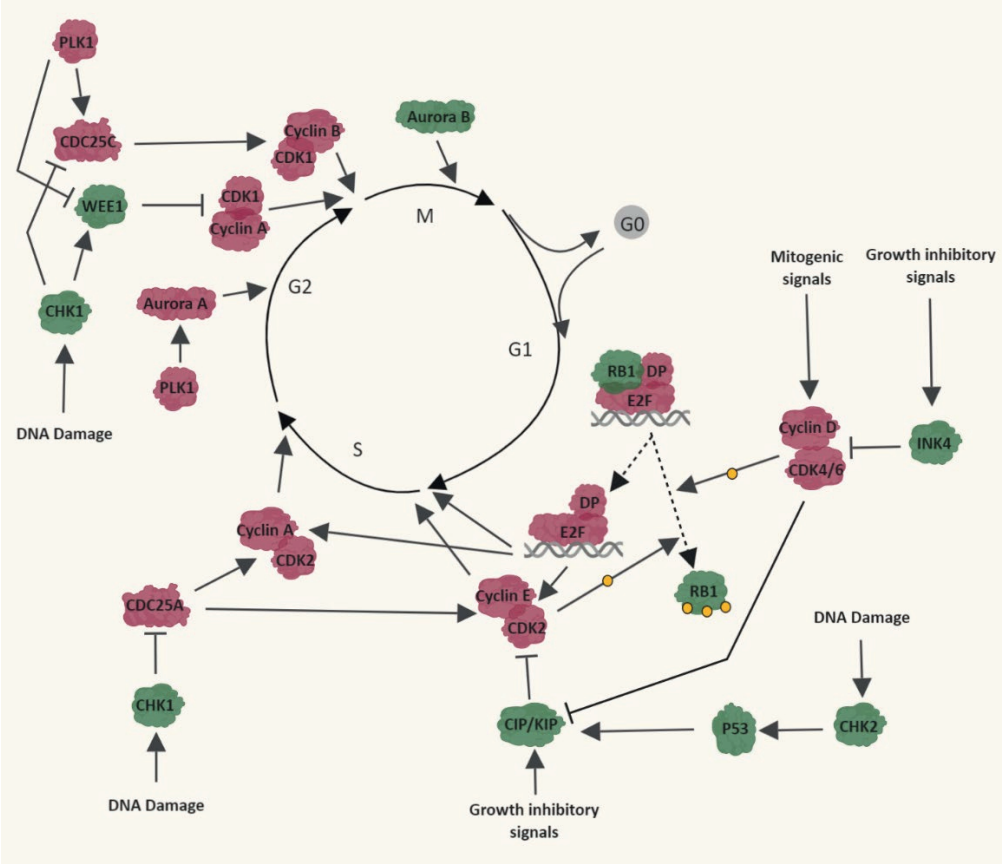

Figure 6. Major proteins involved in cell cycle progression. Activation of cyclin dependent kinases by mitogenic signals results in progression from G1 to S phase. This happens by phosphorylation of retinoblastoma protein 1 (RB1) and subsequent activation of E2F transcription factors. This is negatively regulated by growth inhibitory signals that activate CIP/KIP and INK4, inhibitors of cyclin dependent kinases. Different CDK-cyclin complexes regulate progression through G1-S-G2 and M phase. In addition, several other important proteins such as Polo like kinases (PLK1) and Aurora kinases (Aurora A and B) also regulate cell cycle progression. Cells can exit the cell cycle and go in G0, which can either be reversible of irreversible. DNA damage can trigger cell cycle checkpoints (CHK1 or CHK2), which depends on the type of DNA damage and cell cycle phase. Figure based on [108].

discover new therapeutic targets for chondrosarcoma. For this previously established chondrosarcoma cell lines are used and were chosen based on genetic background and their utility in terms of transfection efficiency. These lines are used to discover new possible targetable vulnerabilities in chondrosarcoma and where possible we tried to validate our findings in more translatable models.

Outline of the thesis

The aim of my thesis was to identify new therapeutic targets for patients with chondrosarcoma. In addition, we tried to explore the biology of chondrosarcoma and tried to find mechanisms that cause chemoresistance in these cells.

Chapter 2 describes the molecular drivers in chondrosarcoma. All known genetic alterations and pathway deregulations are described and discussed in detail for each chondrosarcoma subtype.

In chapter 3 and 4 the role of Bcl-2 family members in chondrosarcoma is studied. **Chapter 3** describes targeting of Bcl-2 family members using ABT-737 as a possible therapeutic strategy in mesenchymal chondrosarcoma, a highly malignant rare chondrosarcoma subtype. In **chapter 4** the role of the different Bcl-2 family members is assessed separately using a selective Bcl-2 and a selective Bcl-xl inhibitor, showing that Bcl-xl is the most promising Bcl-2 family member in targeted treatment of chondrosarcoma.

Screening approaches to identify new therapeutic targets are performed in chapter 5, 6 and 7. **Chapter 5** describes a focussed apoptosis siRNA screen identifying Survivin as a potential therapeutic target for chondrosarcoma patients. The role of kinases is explored in **chapter 6**, where a kinase focussed siRNA screen and compound screen is performed. We show that cell cycle regulators are important in chondrosarcoma cell survival and might be potential targets in a subset of high-grade chondrosarcoma patients. A metabolic compound screen is described in **chapter 7**. Here the role of the mTOR pathway is assessed in chondrosarcoma cell lines and an orthotopic mouse model.

Chapter 8 focusses on radiation resistance in chondrosarcoma. Cell lines and tumour explants are treated with radiotherapy and possible markers predicting radio-sensitivity are assessed using sequencing and immunohistochemistry.

Chapter 9 contains the summary and conclusion and results of different studies will be discussed and put in a broader perspective.

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