Chapter 1

General Introduction

I. Primary Chondrosarcoma

Primary bone tumors are rare, constituting 0.2% of all reported neoplasms (1). Malignant cartilage tumors of bone are classified as chondrosarcomas. After osteosarcoma, primary chondrosarcoma is the second most frequent high grade bone tumor in humans, and is most common in adults between the 3rd and 6th decade of life. Conventional chondrosarcoma is the most common variant, and constitutes about 85% of all chondrosarcomas (fig 1) (2).
Chondrosarcomas frequently occur in the bones of the pelvis, ribs and bones of the extremities, and are much more rare in the skull or small bones of the hands and feet. Patients often present with persistent swelling and/ or pain. Using conventional imaging, a radiolucent lesion with ring-like opacities can be observed due to calcifications, MRI imaging is helpful to determine matrix calcification and soft-tissue involvement. A central chondrosarcoma typically arises in the metaphysis or epiphysis and dynamic contrast enhanced MRI is a helpful tool in identifying malignancy. In the diagnosis of a peripheral chondrosarcoma, arising on the surface of the bone, an MRI scan can be particularly helpful as a cartilage cap >1.5-2cm is an indicator of malignancy (3;4). Histologically, conventional chondrosarcomas are divided into 3 grades (5). As the former grade I chondrosarcoma (CSI) (6) was rarely reported to metastasize, in the 2013 WHO classification CSI is reclassified as atypical cartilaginous tumor (ACT), being of the intermediate, locally aggressive category and will here be referred to as ACT/ CSI. High grade chondrosarcomas that are more prone to metastasize, are classified as grade II (CSII) and grade III (CSIII) chondrosarcoma (2).
An important predictor for local recurrence is the margin status upon resection. The most important predictor of metastases is histological grade. So far no molecular markers have been identified that are independent from and better than histological grading, and correct assessment of histological grade requires an experienced pathologist. An ACT/ CSI will show ample hyaline cartilaginous matrix surrounding the cells that have small hyperchromatic nuclei, whereas CSII and CSIII will show increased cellularity, nuclear atypia, and nuclear size. Grade III lesions are more cellular than grade II lesions with high mitotic count, more myxoid matrix and spindle cell changes at the edge of the lobuli. The distinction between a benign enchondroma and an ACT/ CSI can prove difficult and a high interobserver variability has been established (7-9). For clinical management, the distinction between ACT/ CSI and CSII is more important, which is less subjected to interobserver variability (8). In ACT/ CSI, curettage with local adjuvans is showing good long term results with <6% local recurrence rate (10) and currently has a 5 year survival rate of 83%. In CSII and CSIII, en-bloc resection is required and wide resection margins are necessary to prevent local recurrence, as 13% of local recurrences show an increase in histological grade. The combined 5 year survival rate for CSII/ CSIII is 53% (2;5;10;11).

II. Secondary chondrosarcoma arising in benign cartilaginous tumors

Enchondroma and secondary central chondrosarcoma

A conventional chondrosarcoma arising from a pre-existing benign precursor lesion is classified as a secondary chondrosarcoma (fig 1). Enchondromas are benign cartilaginous lesions arising in the medullary cavity of the bone. Enchondromas are most common in the short tubular bones where they can cause palpable swellings, and less common in the long tubular bones and flat bones, in which case these lesions are mostly asymptomatic and often incidentally detected in radiographs. The majority of enchondromas are detected between the second to fifth decade of life and are solitary. Occasionally involvement of more than one bone or multiple lesions in one bone are observed, indicating enchondromatosis. The most common enchondromatosis subtypes are Ollier disease (multiple enchondromas often in a unilateral distribution) or Maffucci syndrome (multiple enchondromas combined with (spindle cell) hemangiomas) (12;13).

Recently, mutations in *isocitrate dehydrogenase -1* (*IDH1*) or *-2* (*IDH2*) were identified in 87-93% of enchondromas (14-16). IDH mutations were originally discovered in glioblastomas (17), and the molecular mechanism in tumor formation is currently being elucidated. Both IDH1 and IDH2 are mitochondrial enzymes in the tricarboxylic acid (TCA) cycle and are dependent on NADP+. Under normoxic conditions, NADPH and α-ketoglutarate is produced, the latter crucial in preventing the stabilization of HIF-1α (18). IDH mutations lead to a shift in the TCA cycle favoring the production of the oncometabolite D-2-hydroxyglutarate (D2HG) (19-22). The increased D2HG production has been reported to contribute to tumor formation through epigenetic mechanisms such as impairment of histone and DNA demethylation (23-25). As α-ketoglutarate and D2HG show a high degree of structural homology, they share the same substrates. Accumulation of D2HG can inhibit the histone demethylases, as well as the TET family of methylcytosine hydroxylases, otherwise activated by α-ketoglutarate (26-28). Aberrant DNA methylation patterns have been observed in IDH mutant primary tumors (23;28-31) including enchondromas (14). Using a knock-in approach, a heterozygous IDH1 R132H mutation was introduced into a human colorectal cancer cell line, inducing genome-wide histone and CpG methylation, along with a change in gene expression, supporting the causative role of IDH mutations in tumorigenesis (32). Moreover, IDH induced hypermethylation has been shown to block cell differentiation in hematopoetic stem/progenitor cells and adipocytes (24;28).

Prolyl hydroxylases, responsible for the degradation of HIF1/2α proteins and maturation of collagen proteins, are also dependent on α-ketoglutarate. The role of D2HG in creating a "pseudohypoxic" state by preventing the proteosomal degradation of HIF1α is still under debate (27;33), but IDH1 R132H knock-in mutant mice were shown to have decreased ROS levels and stabilization of HIF1α proteins. Though IDH1 mutations were under a brain-specific promoter, the same

study showed collagen maturation to be perturbed after IDH1 R132H knock-in and to be attributed to D2HG accumulation (34).
The role of IDH mutations and the induced epigenetic changes resulting from D2HG accumulation in the progression from enchondroma to atypical cartilaginous tumor/CSI still remains to be determined (fig 1). Enchondromas were found to be mosaic, harboring both wildtype cells and IDH mutant cells (14). Moreover, IDH mutations were found in 38-70% of primary and 85-88% of secondary chondrosarcomas (14;16;35). In enchondromatosis the risk factors for malignant transformation include age and location. Enchondromas located in the long tubular bones and flat bones were reported to give rise to chondrosarcomas in 45% and 50% of Ollier and Maffucci patients, respectively. Although enchondromas are most common at the short tubular bones, malignancy at this location occurred in only 15% of Ollier patients, and in none of the Maffucci patients. Patients with enchondromas in both the long and short tubular bones were most prone to develop chondrosarcomas, showing 46% and 62% incidence in Ollier and Maffucci patients, respectively (2;36).

Osteochondroma and secondary peripheral chondrosarcoma
Secondary peripheral chondrosarcomas arise from osteochondromas, benign cartilage capped bony projections arising on the surface of the bone. Patients usually present in the first three decades of life with a, usually asymptomatic, persistent hard mass. In approximately 15% of patients multiple lesions can be found, characteristic of multiple osteochondromas (previously called multiple hereditary exostoses), a hereditary autosomal dominant syndrome (37) caused by germline mutations in *exostosin-1* (*EXT1*) or *-2* (*EXT2*) (fig 1). In sporadic osteochondromas, somatic homozygous deletions of *EXT1* can be found (37;38). EXT is a glucosyltransferase important in the synthesis of heparan sulfate, a proteoglycan essential for IHH diffusion in the growth plate (39).
Chondrocytes mature in a matrix rich environment and are dependent on the proteoglycan-mediated diffusion of morphogens through their extracellular matrix. The morphogen Indian hedgehog (IHH) and parathyroid hormone-related protein (PTHrP) control the cells in the hypertrophic and proliferating zones, through a negative feedback loop (40;41). Impaired IHH signaling and retention of PTHrP can lead to an increase in proliferating chondrocytes and inhibition of differentiation. Recently, osteochondromas were shown to be mosaic, containing both wildtype and *EXT* mutant cells (42;43), and secondary peripheral chondrosarcomas have been shown to arise from the wildtype *EXT* cells as they no longer contain *EXT* mutations (fig 1) (44). The *EXT* mutations of their neighboring cells, however, are thought to create an environment in which the wildtype cells are prone to developing yet unknown (epi-)genetic changes (fig 1) (45). Though no longer containing *EXT* mutations, loss of inhibitory feedback of IHH on PTHrP and BCL-2 was shown to correlate with progression from osteochondroma to peripheral chondrosarcoma (46;47).

In central chondrosarcoma, EXT and its downstream pathways have been studied as well. Despite wildtype *EXT*, aberrant IHH signaling is found, along with retention of PTHrP in high grade central chondrosarcomas (48;49), suggesting an EXT independent mechanism towards tumorigenesis. Reports about the suitability of the hedgehog pathway as a therapeutic target in central chondrosarcoma are conflicting, as targeting the hedgehog pathway with triparanol led to reduction in tumor growth in mouse xenografts (48), but cyclopamine was effective in only 1 of 6 cell lines (50).
Malignant transformation to secondary peripheral chondrosarcoma is found in ~1% of solitary osteochondromas, whereas in patients with multiple osteochondromas progression to secondary peripheral chondrosarcoma is reported in ~5% of cases (2).

III Rare chondrosarcoma subtypes
The rare chondrosarcoma subtypes include dediffferentiated chondrosarcoma (10%), mesenchymal chondrosarcoma (3%), and clear cell chondrosarcoma (2%).

Dedifferentiated chondrosarcoma
A dedifferentiated chondrosarcoma is histologically comprised of two clearly defined compartments; a highly cellular high grade anaplastic or undifferentiated component juxtaposed to a usually low grade chondrosarcoma. In addition to pain and swelling, patients can present with paresthesia and pathological fractures due to cortical destruction by the tumor. Upon imaging, an ill-defined lesion can be observed, and the irregular calcification showing both ring-like cartilaginous components and a high grade lytic permeable component can indicate the presence of a dedifferentiated component (51). Dedifferentiated chondrosarcoma shows a relentless clinical course and a large retrospective study revealed that survival is highly dependent on presence of metastasis at diagnosis. Patients presenting with metastases have a 2 year survival rate of only 10%, compared to a 28% 10 year survival in patients presenting without metastasis (52).
As dedifferentiated chondrosarcoma histologically shows two clearly defined regions, genetic markers were investigated to determine whether the two compartments are derived from a common origin (53;54). Recently, IDH mutations were found in 54% of dedifferentiated chondrosarcomas, and were not mutually exclusive with TP53 mutations (14;53). As identical IDH and TP53 mutations as well as loss of p16 expression has been found in the two different components this is suggestive of a common clonal origin and thought to be early events contributing to its histogenesis (fig 1) (53;54). Immunohistochemically, distinct pathway activation such as PTHLH expression (55) as well as different loss of heterozygosity patterns (54) can be observed, suggesting an early diversion of the two components (fig 1). MYC (56) and MDM2 (53) amplification have only been

reported in the dedifferentiated component and are therefore likely to be late events occurring after the separation of the two components
As the prognosis is dependent on the dedifferentiated component, research aims at identifying pathways distinctly activated in this component for the development of therapeutic strategies.

Mesenchymal chondrosarcoma
Mesenchymal chondrosarcoma shows a mixture of undifferentiated cells with islands of well differentiated hyaline cartilage. Mesenchymal chondrosarcoma shows a widespread skeletal distribution, including the craniofacial bones, and primary soft tissue localization is reported in approximately 20-30%. Patients present with pain and swelling, occasionally of sudden onset, but most often present for at least one year (57). Due to its aggressive course, long term follow up is recommended. Tumor localization in the jaw or metastasis at time of presentation is a bad indicator for prognosis, whereas young age appears to be a good prognostic factor (58;59). Upon imaging, mesenchymal chondrosarcoma presents as an ill-defined, lytic lesion, often with cortical destruction, in most cases resembling high grade conventional chondrosarcoma (57).
Mesenchymal chondrosarcomas were recently shown to carry the HEY1-NCOA2 fusion gene (60), and a HEY1-NCOA2 negative lesion was found to carry an IRF2BP2-CDX1 fusion gene (fig 1) (61). Mesenchymal chondrosarcomas have not been found to carry IDH mutations, and mutations in TP53, p16, or MDM2 are also found to be rare events (53;62;63). However, Rb pathway dysregulation was found in 70% of mesenchymal chondrosarcomas (53).

Clear cell chondrosarcoma
Clear cell chondrosarcoma represents a low grade chondrosarcoma showing clear cells with a distinct membrane surrounded by a hyaline cartilaginous matrix. Clear cell chondrosarcoma is most common on the epiphyseal ends of long bones, and shows the best prognosis with a mortality of 15%. The most common presenting symptom is pain, and upon imaging a well defined lytic lesion in the epiphysis of a long bone is observed, occasionally showing a sclerotic rim. The best curative option is en-bloc excision with clear margins. Clear cell chondrosarcoma is the only subtype reported to have a sex preference as it occurs three times more often in men than in women (64).
Clear cell chondrosarcomas show random chromosome losses or gains, often with hemizygous involvement of the CDKN2A/p16 locus (fig 1). No involvement of IDH, TP53, or MDM2 has been reported (14;53).

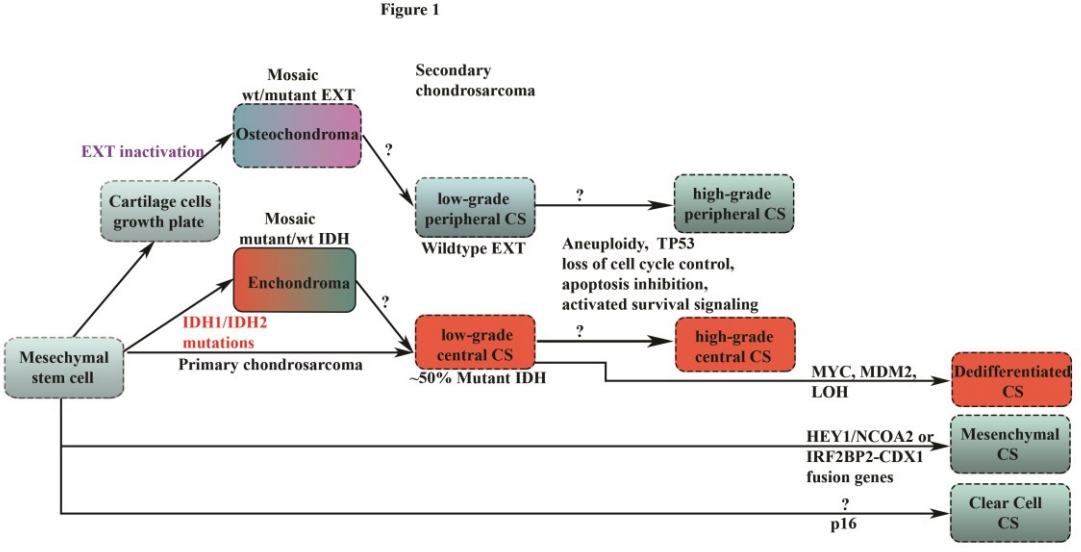

Figure 1.1. Progression model for conventional chondrosarcoma. A: In contrast to secondary chondrosarcoma, primary chondrosarcoma arises from the precursor cell without an intermittent benign lesion. EXT inactivation is observed in the benign osteochondroma. Peripheral chondrosarcomas are wildtype for EXT, and arise from the EXT wildtype cells in the osteochondroma. IDH mutations are found in enchondromas as well as low and high grade central chondrosarcomas, but only enchondromas were shown to be mosaic. Approximately 50% of low grade chondrosarcomas were shown to harbor IDH mutations. The (epi-)genetic events leading to progression from benign precursor lesion to a low grade tumor are unknown, and in progression from low grade to high grade, aneuploidy, loss of heterozygosity, as well as deregulations in the apoptosis, survival and growth plate signaling pathways are found. All these factors are likely to contribute to progression in grade and resistance to therapy. Both the cartilaginous and dedifferentiated components of dedifferentiated chondrosarcoma have been shown to harbor IDH and TP53 mutations, supporting the hypothesis of a common clonal origin. MYC and MDM2 mutations as well as loss of heterozygosity (LOH) patterns were found to differ between components, suggesting occurrence after component separation. In mesenchymal chondrosarcoma, the HEY1/NCOA2 fusion gene was recently identified, and the IRF2BP2-CDX1 fusion gene in a HEY1/NCOA2 negative lesion, indicating the mutual exclusive nature of these fusion genes. In clear cell chondrosarcoma, other than common loss of p16 protein expression, no causative mutations have been identified as of yet.

III. Chondrosarcoma model systems

Recurrent and metastatic disease as well as chondrosarcomas located at unresectable sites present a major problem, as conventional chemo- and radiotherapy have shown to be ineffective (64). In order to improve survival rates and quality of life, there is an urgent need for new therapeutic strategies in chondrosarcoma. Chemoresistance has long been attributed to the cartilaginous matrix surrounding the cells, to the relatively low mitotic rate, as well as to possible expression of multi-drug resistance genes. Increasingly, activation of various pathways are being recognized in prohibiting apoptosis and promoting cell survival in chondrosarcoma (65).

Ever since the discovery of Imatinib (11), as the prototype of targeted therapy in solid tumors, there has been an explosive growth in targeted therapies. In the preclinical search for the proper treatment strategy for chondrosarcoma, adequate model systems are invaluable. This is especially important in a rare malignancy such as chondrosarcoma, in which it is difficult to conduct large randomized clinical trials and for which it can be challenging to obtain funding from the pharmaceutical industry.

Chondrosarcoma cell lines

In cancer research, the use of cancer cell lines facilitates the study of the characteristics and behavior of cancer cells. Since the publication of the first human cell line in 1952, HeLa (66), the changes in the field of tissue culture have rapidly advanced cancer research. However, through HeLa, the community also learned about the pitfalls of cell lines. The rapid and wide dissemination of HeLa shortly after its discovery led to large scale cell line cross contamination. Today, most labs have implemented cell line typing in order to closely monitor and maintain the unique identity of each cell line (67).

Cell lines prove an especially useful tool in the search for new treatment strategies. A first step is to investigate the effect of any treatment on cell viability using basic characteristics of the cells, such as presence of ATP or mitochondrial activity. However, a limitation of such viability assays can be the lack of proof of occurrence of apoptosis. In a healthy cell, the anionic phosphatidylserines are facing the cytoplasmic side of the plasma membrane. During apoptosis, disruption of phospholipids in the cellular membrane will cause the phosphatidylserines to face outwards. Being a natural binding site for human AnnexinV, recombinant AnnexinV can be used in combination with a fluorescent label such as FITC to detect apoptosis in cells (68). Combined with live cell imaging, specific apoptosis occurrence after drug exposure can be monitored over time (69).

Real-time monitoring of cell number can also be performed with the xCelligence system (ACEA Biosciences). Providing a label-free system, based on cell density, this system uses small gold electrodes which cover the bottom of the plate. The electrodes are connected to a computer outside the incubator and as cells multiply, an increase in resistance over the electrodes can be converted into a cell index (70). Moreover, the xCelligence provides a real-time migration assay, with a 16 well-

plate containing micropores. As cells are plated on the side without electrodes, a signal will only arise once cells have started migrating (71).
Cell lines, however, are so-called 2D cultures. As chondrosarcomas histologically show cartilaginous matrix surrounding the cells, a 3D culturing system to investigate its role can be used. In 3D culturing, cells can be grown in scaffolds made to resemble the natural extracellular matrix (ECM). Correctly mimicking the ECM and controlling appropriate stimuli such as growth factors can be challenging in a system using scaffolds. A second method, widely used in oncology to examine tumor behavior, are multicellular spheroids. In this method, various approaches are applied to obtain cell spheroids, which are maintained using appropriate growth factors (72). As chondrocytes (73) were shown to create matrix in 3D culture, this model can be used to model chondrosarcoma behavior and drug response *in vivo*.

Chondrosarcoma mouse models
Ideally, drugs proven effective in cell lines are subsequently proven effective *in vivo* in rodent models before proceeding to the clinic. A stable, reproducible mouse model is advantageous for standardizing *in vivo* research. Current chondrosarcoma mouse models either show subcutaneous xenografting of tumor tissue immediately after resection from the patient, or subcutaneous injection of cell lines (74). An orthotopic model, more closely resembling the patient situation would be preferable. The pharmacokinetics of a drug need to be taken into account, as the distribution to an intra-osseous and a subcutaneous tumor can differ. In addition, subcutaneous injection could alter chondrosarcoma cell behavior through communication with surrounding cells. An orthotopic mouse model is therefore more likely to correctly predict response of the primary tumor than a subcutaneous model.
In the literature several chondrosarcoma rodent models exist. One such model is the orthotopic swarm rat chondrosarcoma model, created using cell lines derived from a chondrosarcoma that spontaneously arose in a sprague-dawley rat. However, it was recently shown that the cell lines used to create the model consist of different cytogenetic properties (75). The literature also describes an orthotopic chondrosarcoma mouse model, derived from the JJ012 cell line (76). A limitation of these methods is that the role of EXT or IDH mutations in the tumorigenesis of osteochondromas and enchondromas, respectively, cannot be investigated. Conditional *Ext1* knock out mouse models for EXT exist, and also show exostosis formation, closely resembling human osteochondromas, however, no malignant transformation to chondrosarcoma is observed in these lesions (77-79). So far, two *IDH* conditional knock-in mutant mouse model exist, specific for neural or hematopoetic progenitor cells, and in neither model has enchondroma or chondrosarcoma formation been found (34;80). As tumor size can only be assessed at time of animal sacrifice, when testing drug response, a method enabling live monitoring of tumor growth would be ideal. Using luciferase constructs, tumor growth can be monitored during the experiment with minimal stress to the animal,

as a single intra-peritoneal injection is required to activate the construct and the tumor can be visualized using a bio-imager (81).

V. Approaches to identify new treatment strategies

The hallmarks of cancer as characterized by Weinberg and Hanahan in 2000 (82), identified pathways distinctive to cancer cells which can be utilized when designing cancer therapeutics. Two of these pathways are recognized to be the apoptosis and survival pathways. Apoptosis pathways lead to an acquired capacity to evade programmed cell death. Survival pathways including tyrosine kinases can lead to a self-sufficiency in growth signals, as well as insensitivity to anti-growth signals, limitless replicative potential, sustained angiogenesis, and the acquired ability to invade distant tissue and metastasize(83;84).

Apoptotic pathways

One class of apoptotic proteins is the Bcl-2 family, and a shift favoring anti-apoptotic Bcl-2 proteins can lead to the acquired capacity to evade programmed cell death, even in the presence of death signals (fig 2).

Besides from its anti-apoptotic properties, Bcl-2 is also a player in the indian hedgehog (IHH) pathway during endochondral bone development (85). In both central and peripheral chondrosarcoma aberrant IHH signaling is observed along with retention of PTHrP. Apart from inhibiting differentiation, PTHrP also inhibits apoptosis through stimulating the expression of the anti-apoptotic protein Bcl-2, found to be overexpressed in high grade chondrosarcomas (fig 1) (46;48;49;85-89).

The four Bcl-2 homology (BH) domains are characteristic of the Bcl-2 family, enabling oligodimerization. Three classes of proteins control cell survival and regulate apoptosis. Anti-apoptotic (Bcl-2, Bcl-xl, Bcl-w) and pro-apoptotic (BAX, BAK) family members possess all four BH domains, creating a hydrophobic groove able to bind BH3-only proteins (90). Two classes of BH3 proteins exist: i) activating BH3 proteins (BID, BIM) promote the oligomerization of BAX/BAK dimers, and ii) sensitizing BH3 proteins (BAD) bind the anti-apoptoptic proteins (fig 2). Only activating BH3-only proteins have the ability to induce cytochrome C release, and can in turn be released from the anti-apoptotic proteins by the sensitizing BH3-only proteins. The upregulation of anti-apoptotic proteins as reported in cancer, leads to the sequestration of BH3-only proteins, and prohibits the formation of BAX/BAK dimers (fig 2). Without the insertion of BAX/BAK dimers, the mitochondrial membrane will not be permeabilized, with the consequential lack of cytochrome C release and caspase activation (90;91).

Inhibitors of the Bcl-2 family are based on protein-protein interactions. The first inhibitor to be discovered was HA14-1, a nonpeptidic ligand of Bcl-2 (92). The new class of inhibitors are called BH3 mimetics, specifically binding the hydrophobic BH3 groove of anti-apoptotic Bcl-2 proteins. The BH3 mimetic ABT-737 (93) has shown promising preclinical results and its orally available counterpart, Navitoclax (ABT-263) (94), is currently in clinical trials for various

malignancies, including solid tumors (95). Resistance to ABT-737 has been attributed to increased Mcl-1 expression (96), and recently, a BH3 alpha-helix mimetic was designed, JY-1-106, specifically disrupting the protein-protein interactions between Bcl-xl and Mcl-1 with Bak (97). Finally, the BH3 mimetic GX15-070, obatoclax, has been developed to bind Bcl-2, Bcl-xl, Bcl-w, and MCL-1 (98) and is currently in clinical trials.

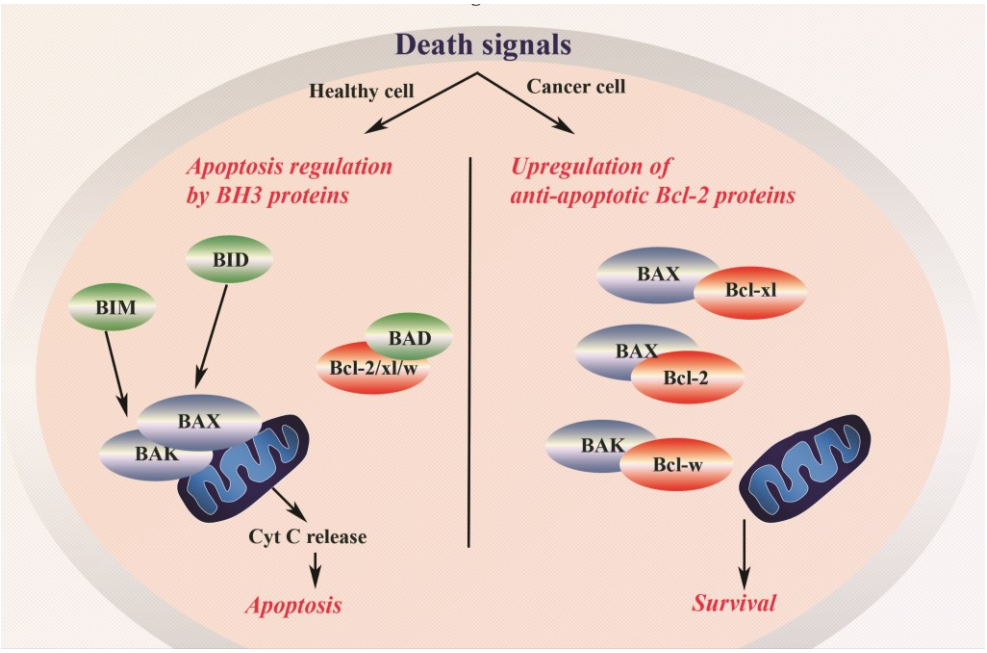

Figure 1.2. Apoptosis pathway. In a healthy cell (left panel), response to death signals such as DNA damage can occur through activation of BH3 only proteins. Two types of BH3 only proteins will allow for apoptosis to occur: sensitizing BH3 only proteins like BAD will release BAX and BAK from the anti-apoptotic Bcl-2 proteins, Bcl-2, Bcl-xl, and Bcl-w. The activating BH3 only proteins, such as BID or BIM, will then promote BAX/BAK heterodimerization and insertion into the mitochondrial membrane, upon which the mitochondrial membrane becomes permeabilized. Consequentially, cytochrome C and calcium are released and caspases are activated, leading to apoptosis. In cancer, upregulation of anti-apoptotic proteins is often found (right panel), in which case a cell becomes desensitized to the activation of BH3 only proteins. BAX and BAK remain sequestered to anti-apoptotic proteins and the mitochondrial membrane is not permeabilized. The upregulation of anti-apoptotic proteins hereby leads to survival of cells, even in the presence of death signals, such as they might occur after chemotherapy.

Survival pathways
In biochemistry, an enzyme actively transferring phosphate groups from proteins in the process of phosphorylation is called a kinase. Tyrosine kinases are kinases which become activated once phosphorylated at a specific catalytic tyrosine site (99). Of the hallmarks of cancer (82), self sufficiency of growth signals, limitless replicative potential and tissue invasion can be traced back to tyrosine kinases which play an important role in survival pathways governing cancer cell survival, proliferation, and metastasis (83;84). Using kinome profiling, tyrosine kinase pathways were found to be active in chondrosarcoma (100).
Receptor tyrosine kinases (RTK) have a cytoplasmic tyrosine kinase domain, and an extracellular ligand binding regulatory domain. As a rule, RTKs require ligand binding (typically growth factors) to undergo a conformational change allowing for dimerization and autophosphorylation, with resulting downstream signaling (101). The increased RTK signaling observed in cancer can occur through gene amplification leading to constitutive activation, or through close clustering on the membrane leading to homodimerization in absence of ligand (92). The importance of tyrosine kinase signaling in cancer has led to the development of a multitude of different tyrosine kinase inhibitors, of which 18 have now been approved by the FDA for a variety of malignancies (102).

VI. Aim and outline of the thesis

The aim of this thesis was to unravel mechanisms for chemoresistance and identify new therapeutic strategies for targeted treatment in chondrosarcoma. In order to rapidly translate results from basic research to clinical practice, pre-clinical models including chondrosarcoma cell lines and mouse models were developed.
Chapter 2 focuses on new therapeutic strategies in chondrosarcoma. As such a review of recent preclinical research is linked to ongoing or completed clinical trials in chondrosarcoma in an attempt to provide a concise overview of the current state of the art of possibilities for targeted therapy in chondrosarcoma. In light of the recent discovery of IDH mutations, and advances regarding EXT mutations, their respective roles in chondrosarcoma tumorigenesis and chemoresistance is discussed, as well as the therapeutic potential of hedgehog signaling.
In **chapters 3 and 4** the development of new chondrosarcoma model systems is described. First, the generation and characterization of three new chondrosarcoma cell lines (**chapter 3**), and second, the development of an orthotopic chondrosarcoma mouse model (**chapter 4**).

In **chapters 5-8**, mechanisms of chondrosarcoma chemoresistance are investigated. **Chapter 5** explores chemoresistance mechanisms in conventional chondrosarcoma, focusing on drug inaccessibility due to matrix hindrance, activity of multidrug resistance transporters, and overexpression of anti-apoptotic Bcl-2 proteins (45;94). The role of Bcl-2 proteins in chemoresistance of conventional (**chapter 5**) and dedifferentiated chondrosarcoma (**chapter 6**) is further examined using the BH-3 mimetic ABT-737 in combination with doxorubicin or cisplatin. As rare chondrosarcoma subtypes histologically resemble different stages of the growth plate, in **chapter 6**, immunohistochemistry is performed on tissue microarrays of rare chondrosarcoma subtypes, to examine expression levels of proteins involved in growth plate signaling pathways.
Finally, tyrosine kinase signaling pathways are investigated. Using kinome profiling, the PI3K/AKT and SRC pathway were found to be active (100), and were hypothesized to play a role in chondrosarcoma chemoresistance. In **chapter 7** we therefore combine enzastaurin (AKT inhibition) and dasatinib (SRC inhibition) with doxorubicin. As Src kinases are reported to play a role in metastasis formation (95), migration assays are performed. Hypothesizing that an upregulation in protein tyrosine kinases could well be concomitant with an upregulation of receptor tyrosine kinases, in **chapter 8** a similar approach is used to investigate the activities of receptor tyrosine kinases (RTK) in chondrosarcoma cell lines using a phospho-receptor tyrosine kinase array. The roles of RTK activation in chondrosarcoma proliferation is further examined using kinase inhibitors, with analysis of downstream pathway activation. In comparison, mTOR pathway activation is studied to investigate downstream activation of RTK signaling.
Chapter 9 will conclude with a summary of the results and an outlook to the future.

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