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

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Chapter 10

Summary

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Summary

Chondrosarcomas are malignant cartilage producing tumours representing 20% of malignant bone tumours [1]. They predominantly develop in the bones of the pelvis, ribs and extremities and are diagnosed in a broad age range, but most commonly around the age of fifty. Chondrosarcoma can be subdivided in conventional chondrosarcoma (85%), dedifferentiated chondrosarcoma (10%) [2], mesenchymal chondrosarcoma (2%) [3], clear cell chondrosarcoma (2%) [4] and periosteal chondrosarcoma (1%) [1]. Conventional chondrosarcoma is further subdivided into central (85%) and peripheral (15%), based on its location in the medulla or next to the bone. Their histological appearance is similar however they show a different genetic background; central chondrosarcomas present with mutation in *IDH1* or *IDH2* in +/- fifty percent of the cases while peripheral chondrosarcomas show alterations in *EXT1* and *EXT2* genes. Conventional chondrosarcoma can be subdivided in three different grades based on its histological appearance (Grade I-III). Grade III chondrosarcomas are most malignant and show a 10 years survival rate of 26-29% [1, 5-7]. Dedifferentiated chondrosarcoma is a high grade tumour showing *IDH1* or *IDH2* mutations in approximately fifty percent of the cases [8]. It has a very low 10 years survival rate of only 10-28% depending on the presence of metastasis [9]. Mesenchymal chondrosarcomas are genetically characterized by a *HEY1-NCOA2* fusion, and 10 years survival rates are reported between 27 and 67% [10, 11]. Clear cell chondrosarcomas are low grade tumours with a mortality rate of 15% [12]. No recurrent initiating genetic alterations have been identified in this subtype. Periosteal chondrosarcomas are very rare and few studies have been reported investigating this subtype. *IDH1* or *IDH2* mutations have been identified in 15% of the cases [13].

Treatment of chondrosarcoma patients is mainly by surgery, since tumours are relatively resistant toward chemo-and radiotherapy. Several possible mechanisms are hypothesized why chondrosarcoma cells are therapy resistant, including upregulation of multi-drug resistance pumps and anti-apoptotic proteins, but likely up regulation of other pro-survival mechanisms and down regulation of pro-cell death mechanisms are playing a role [14-17].

The aim of this thesis was to look further into these mechanisms and find alternative targetable treatment options for chondrosarcoma patients by using a screening based approach. In addition we investigated the role of Bcl-2 family members and its use in targeting the apoptosis pathway in

chondrosarcoma. Furthermore possible mechanisms of radio-resistance were investigated in chondrosarcoma cell lines and explants. In **Chapter 2** a general overview is given about genetic alterations and pathway deregulations that have been identified in the different chondrosarcoma subtypes. This illustrates the complexity and heterogeneity of the different chondrosarcoma subtypes. **Chapter 3** describes the characterization of a new mesenchymal chondrosarcoma cell line MSC170, which is the first cell line that has been reported of this rare chondrosarcoma subtype. We confirmed the presence of the characteristic HEY1-NCAO2 fusion. Expression of Bcl-2 and especially Bcl-xl was present in this cell line and inhibiting Bcl-2 family members using ABT-737 resulted in synthetic lethality with doxorubicin as well as cisplatin as was previously shown for conventional and dedifferentiated chondrosarcoma [15, 16]. **Chapter 4** dives deeper into the role of Bcl-2 family members in chondrosarcoma and resistance towards conventional chemotherapy. We looked into single inhibition of Bcl-2 and Bcl-xl specifically in chondrosarcoma cell lines and in a Swarm Rat Chondrosarcoma (SRC) model. Interestingly we found that not Bcl-2, but Bcl-xl is most important for chondrosarcoma survival *in vitro* as well as *in vivo*. A subset of cell lines showed a synergistic response when Bcl-xl was inhibited in combination with chemotherapy, indicating that involvement of Bcl-xl in chemotherapy resistance is heterogenous across the panel of chondrosarcoma cell lines that we studied. This shows that further research, and possibly personalized approaches, are needed to assess applicability of combination treatments using Bcl-xl inhibitors in combination with chemotherapy in chondrosarcoma patients. **Chapter 5** describes the discovery of Survivin as an essential regulator in chondrosarcoma. A siRNA screen targeting 51 apoptosis related genes was performed, and identified *BIRC5*, encoding the Survivin protein as essential for chondrosarcoma survival. Survivin has multiple functions depending on its location in the cell [18]. Nuclear Survivin has a role in the cell cycle, while cytoplasmic Survivin is involved in apoptosis. We found nuclear as well as cytoplasmic Survivin expression to be correlated with an increased histological grade. In addition, nuclear Survivin expression was correlated with P53 expression. Of note, *TP53* mutant chondrosarcoma cell lines were especially sensitive for Survivin inhibition, with no caspase dependent apoptosis, but a deregulated cell cycle profile. This shows that Survivin might be a potential therapeutic option for patients with *TP53* mutated chondrosarcoma. In **Chapter 6** the discovery of cell cycle proteins as possible therapeutic targets for chondrosarcoma is described. A kinase focussed siRNA (n=779) and compound screen (n=273) have been performed

in chondrosarcoma cells, that resulted in the discovery of PLK1, CHK1 and AURKA as important targetable kinases of chondrosarcoma cell survival. Kinase inhibitors targeting these three cell cycle regulators were validated in a panel of chondrosarcoma cell lines and dose dependent decreases in viability were observed as well as increased sensitivity towards conventional chemotherapy after inhibition of CHK1. Expression of AURKA and CHK1, but not PLK1 was observed in chondrosarcoma tissue samples, raising the question of the importance of PLK1 as a therapeutic target in chondrosarcoma. A worse overall survival was observed in patients with higher CHK1 expression indicating that high CHK1 expression is correlated with a more malignant phenotype. Although more studies are needed to confirm these findings, this study points to CHK1 as the most promising targetable kinase for treatment of chondrosarcoma patients. **Chapter 7** focusses on the chondrosarcoma metabolome and its vulnerabilities. A metabolic compound screen was designed and 39 compounds targeting the most important metabolic pathways were included. This screen was executed in three different chondrosarcoma cell lines, using four different concentrations of each compound. Six compounds were selected for metabolic profiling resulting in the selection of a dual mTORC1 and mTORC2 inhibitor sapanisertib as most promising hit. Treatment with sapanisertib resulted in a reduction in glycolytic as well as oxidative metabolism and a dose dependent decrease in viability. In addition, tumour growth was slowed down in an orthotopic chondrosarcoma mouse model, indicating that inhibiting mTOR as single or combination treatment might be a potential option for chondrosarcoma treatment. **Chapter 8** shows a possible role for the RB1 pathway in radiotherapy resistance in chondrosarcoma. Chondrosarcoma cell lines showed variable resistance towards γ -radiation. Contradicting previous results [19], chondrosarcoma cell lines treated with mutant IDH1 inhibitor AGI-5198 did not show differential sensitivity towards γ -radiation, nor a difference in glutathione levels. Chondrosarcoma explants treated with γ -radiation showed a variable response pattern, as measured by γ -H2Ax foci quantification. Explants that showed less foci, which were considered more radioresistant, showed alterations in the RB1 pathway, while these alterations were absent in explants with more foci. Further investigation should be performed to determine if patients can be selected for treatment with radiotherapy based upon RB1 pathway status.

In conclusion, this thesis shows the identification of several lead targets that might be useful as targeted treatment options for chondrosarcoma patients. Future research should focus more on the therapeutic value of these targets

in a clinical setting. Novel upcoming techniques, such as CRISPR screening and 3D models that more closely resemble the patient situation, will improve the selection of targetable pathways and will lead to a better translation of pre-clinical research to clinical practise.