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

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Chapter 3

Inhibition of Bcl-2 family members sensitizes mesenchymal chondrosarcoma to conventional chemotherapy; report on a novel mesenchymal chondrosarcoma cell line

This chapter is based on the publication:
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Abstract

Mesenchymal chondrosarcomas are rare and highly aggressive sarcomas occurring in bone and soft tissue, with poor overall survival. Bcl-2 expression was previously shown to be up regulated in mesenchymal chondrosarcomas. We here report on a newly derived mesenchymal chondrosarcoma cell line, MCS170, in which we investigated treatment with the BH3 mimetic ABT-737 alone or in combination with conventional chemotherapy as a possible new therapeutic strategy.

The presence of the characteristic *HEY1-NCOA2* fusion was confirmed in the MCS170 cell line using FISH, RT-PCR and sequencing. The MCS170 cell line was treated with ABT-737 alone or in combination with doxorubicin or cisplatin. Cell viability and proliferation was determined using WST-1 viability assays and the xCELLigence system. Expression of Bcl-2 family members was studied using immunohistochemistry. Apoptosis was determined using the caspase-glo 3/7 assay and Western blot for PARP cleavage.

The MCS170 cell line was sensitive to doxorubicin treatment with an IC_{50} of 0.09 μM after 72 hours, but more resistant to cisplatin treatment with an IC_{50} of 4.5 μM after 72 hours. Cells showed little sensitivity towards ABT-737 with an IC_{50} of 1.8 μM after 72 hours. Combination treatments demonstrated ABT-737 synergism with cisplatin as well as doxorubicin as shown by induction of apoptosis and reduction in cell proliferation.

Restoration of the apoptotic machinery by inhibition of Bcl-2 family members sensitizes MCS170 mesenchymal chondrosarcoma cells to conventional chemotherapy. This indicates that combining the inhibition of Bcl-2 family members with conventional chemotherapy can be a possible therapeutic strategy for patients with mesenchymal chondrosarcoma.

Introduction

Chondrosarcomas are cartilage forming tumors accounting for 20% of all the primary bone malignancies [1]. Chondrosarcoma consists of different subtypes with conventional chondrosarcoma being the most common (75%). More rare chondrosarcoma subtypes include dedifferentiated (10%) [2], mesenchymal (3%) [3] and clear cell chondrosarcoma (2%) [4]. Patients with chondrosarcoma are mainly treated by surgical removal of the tumor, because chemo- and radiotherapy are generally ineffective [5].

Mesenchymal chondrosarcoma is an aggressive, high grade chondrosarcoma subtype with a reported 10 year survival rate between 27 and 67% [6-8]. Recently the survival of mesenchymal chondrosarcoma was determined using the Surveillance, Epidemiology, and End Results (SEER) database, showing that in a group of 205 patients the 10 years survival was 43% [9]. Genetically, it is characterized by a specific characteristic gene fusion between the bHLH domain of *HEY1* (Hes-Related Family BHLH Transcription Factor with YRPW Motif 1) and the C-terminal transcriptional activation domains of *NCOA2* (Nuclear Receptor Coactivator 2) [10]. Fusions involving NCOA2 have also been identified in other soft tissue tumor subtypes including spindle cell rhabdomyosarcoma (*SRF-NCOA2* and *TEAD1-NCOA2*) [11] and soft tissue angiofibroma (*AHRR-NCOA2*) [12] as well as in acute myeloid leukemia (*ETV6-NCOA2 [13]* and *MYST3-NCOA2* [14])

Histologically mesenchymal chondrosarcoma is characterized by variable amounts of differentiated cartilage admixed with small round undifferentiated cells [3]. In 1983 Huvos et al. described that patients with mesenchymal chondrosarcoma in which the small cell component predominated responded better to combination chemo- and radiotherapy [8]. In a more recent study, investigating the effect of chemotherapy in 113 mesenchymal chondrosarcoma patients, it was shown that treatment with chemotherapy reduces the risk of recurrence and improves overall survival in patients with localized disease [7]. Also in previous smaller case reports, patients with mesenchymal chondrosarcoma treated with chemotherapy showed a better survival compared to untreated patients [6, 15-18]. However, in a recent systematic review of 18 studies including 107 patients no correlation between anthracycline based chemotherapy treatment and survival was found [19].

Conventional chondrosarcomas are resistant to chemotherapy. Low grade chondrosarcomas consist of a large amount of cartilage matrix and slowly dividing cells, which was for long thought to be the cause of their

insensitivity to chemotherapy. However, high grade chondrosarcomas have less matrix and more rapidly dividing cells, and are also resistant to chemotherapy. Expression of multidrug resistance pumps has been shown on chondrosarcoma cell lines and patient tissues [20-22]; however this does not prevent doxorubicin from accumulating in the cell nuclei, indicating other mechanisms are involved in chondrosarcoma chemoresistance [21].

Our group has previously shown that Bcl-2 family members play a key role in chemoresistance in conventional and dedifferentiated chondrosarcoma [21, 23]. Pro- and anti-apoptotic proteins of the Bcl-2 family play important roles in initiation of the intrinsic routes of apoptosis, changing the balance from anti- to pro-apoptotic, and activating Bak and Bax to facilitate mitochondrial outer membrane permeabilization. Anti-apoptotic proteins, such as Bcl-2, Bcl-xL and Bcl-w, prevent apoptosis by binding to pro-apoptotic proteins, thereby impeding Bak and Bax activation [24]. Treatment of different chondrosarcoma cell lines with an inhibitor of the Bcl-2 family, including Bcl-2, Bcl-xL and Bcl-w (ABT-737) in combination with doxorubicin or cisplatin resulted in a synergistic effect, as was determined by viability and apoptosis assays [21].

Since we previously demonstrated high expression of Bcl-2 and Bcl-xL in a panel of 23 mesenchymal chondrosarcomas [23], we hypothesized that Bcl-2 family members are also important in mesenchymal chondrosarcoma. So far, however, no mesenchymal chondrosarcoma cell lines were available for functional studies. We here report a novel mesenchymal chondrosarcoma cell line, MCS170, carrying the characteristic HEY1-NCOA2 fusion product, in which we functionally evaluated the role of Bcl-2 family members in chemosensitivity.

Material and methods

Compounds

The BH3-mimetic ABT-737 (S1002, Selleckchem, Munich, Germany) is an inhibitor of Bcl-2, Bcl-xL and Bcl-w and was dissolved in DMSO to a stock concentration of 20 mM. Doxorubicin (DXR) and cisplatin (CDDP) were obtained from the in-house hospital pharmacy in a 0.9% NaCl solution in a 1mg/ml concentration. Z-vad-FMK (550379, BD biosciences, San Jose, CA, USA) was used as a general caspase inhibitor and dissolved in DMSO to a stock concentration of 20 mM.

Cell culture

The MCS170 cell line was generated from a recurrent mesenchymal chondrosarcoma of the spine resected from a 33 year-old man at the department of Pathology, Brigham and Women's Hospital and Harvard Medical School located in Boston, USA and was cultured in IMDM medium (Gibco, Invitrogen Life Technologies, Scotland, UK) supplemented with 15% heat inactivated fetal calf serum (Gibco, Invitrogen Life Technologies, Scotland, UK) and 1% Penicillin-streptomycin (100 U/ml) (Gibco, Invitrogen Life Technologies, Scotland, UK). Cells were grown at 37 °C in a humidified incubator with 95% air and 5% CO_2. Cells were confirmed bi-monthly for absence of mycoplasm infection and cell line identity was confirmed before and after experiments by STR profiling using the GenePrint10™ System from Promega (Promega Benelux BV, Leiden, The Netherlands).

Molecular analysis

The presence of the characteristic *HEY1-NCOA2* fusion between exon 4 of *HEY1* and exon 13 of *NCOA2* was confirmed on RNA isolated using TRIzol (Invitrogen, Carlsbad CA, USA) from the MCS170 cell line and a previously published mesenchymal chondrosarcoma patient sample as a positive control according to methods described previously [10]. Products were purified using MinElute 96 UF PCR purification kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's instructions and Sanger sequenced by Macrogen (macrogen.com).

Fluorescent in situ hybridization was performed as described previously [25] using labeled BAC clones RP11-152C15 and RP11-888F10 [10]. MCS170 cells were screened for mutations in 50 frequently mutated cancer related genes using the Ion AmpliSeq™Cancer Hotspot Panel v2 (Life Technologies, Thermo Fisher Scientific, USA, catalog number 4475346) according to the manufacturer's instructions.

Cell viability assay

When cells reached 75-80% confluence they were washed with PBS, trypsinized, and counted using a Burker Turk counting chamber. MCS170 cells were plated in 96 well plates (10.000 cells/well) and allowed to adhere overnight. Cells were incubated with concentrations of ABT-737, doxorubicin or cisplatin ranging between 0.1 μM to 100 μM for 24, 48 and 72h after which a WST-1 assay was performed according to the

manufacturer's instructions (Roche diagnostics GmbH, Penzberg, Germany). Combination assays were performed 72h after simultaneous addition of ABT-737 (0, 125, 500 nM or 1 μM) and doxorubicin (0, 50, 125 nM or 1 μM) or cisplatin (0, 500 nM, 1 or 5 μM). Results of the WST-1 assay were measured at 450 nm using a spectrophotometer (Victor3V, 1420 multilabel counter, Perkin Elmer, Netherlands). All viability assays were performed in triplicate and at least three times.

Cell proliferation measurement

The RTCA xCELLigence system (Roche Applied Sciences, Almere, the Netherlands) was used to measure cell proliferation in real time. MCS170 cells were plated at a density of 15.000 cells/well, 20 hours before addition of either ABT-737 (1 μM), doxorubicin (125 nM or 1 μM) or cisplatin (5 μM) or the combination between ABT-737 with doxorubicin or cisplatin. After 96 hours the measurement was stopped and the results were analyzed using RTCA software. Experiments were performed in duplicate and repeated at least three times.

Immunohistochemistry

Protein expression of Bcl-2 (Dako, 124), Bcl-xL (Cell signaling clone 54H6) and Bcl-w (Abcam 6C1) was determined using immunohistochemistry on paraffin embedded cell pellets of MCS170 cells. Tonsil was used as a positive control for Bcl-2, kidney for Bcl-xl and cerebellum for Bcl-w. Immunohistochemistry was performed according to standard laboratory methods, using citrate (pH 6) as antigen retrieval method, as previously described [26].

Western blot

PARP (clone 46D11, Cell Signaling Technology, Leiden, the Netherlands) cleavage was determined after 24 hours of compound treatment. Cells were treated with 1 μM ABT-737, 125 nM doxorubicin, 5 μM cisplatin or a combination of ABT-737 and doxorubicin or cisplatin. As a loading control α-tubulin (clone DM1A, Cell Signaling Technology, Leiden, the Netherlands) expression was determined. Cells were harvested using hot SDS buffer (1% SDS, 10 mM Tris/EDTA with complete inhibitor and phosSTOP). Of each sample, 20 μg protein was separated by using SDS-PAGE and transferred to PVDF membranes. Blocking was performed using 5% BSA in PBS/ 0.1%

Tween solution. Membranes were incubated with primary antibody overnight at 4 degrees Celsius, followed by HRP labelled secondary antibody for 30 minutes at room temperature. Blots were developed using ECL solution (Pierce™ ECL Western Blotting Substrate, Fisher Scientific, Landsmeer, the Netherlands).

Apoptosis assay

Apoptosis was detected using the caspase-glo 3/7 assay (Promega, Madison, USA) according to the manufacturer's instructions. In brief, 10.000 MCS170 cells were plated into white walled 96 well plates (Corning B.V. Life Sciences, Amsterdam, the Netherlands) and the following day cells were treated with either 1 μM ABT-737, 125 nM or 1 μM doxorubicin, 5 μM cisplatin or a combination of ABT-737 and doxorubicin or cisplatin for 24 hours. Z-vad treatment was used as a control. Thereafter the substrate provided with the kit was added in a 1:1 dilution and incubated for 30 minutes at room temperature. Luminescence was measured using a luminometer (Victor3V, 1420 multilabel counter, Perkin Elmer, Netherlands). In parallel a viability assay was performed by using the method described in the viability assay section.

Statistical analysis

To evaluate synergy between treatment combinations the Bliss independence model was used. In this model the assumption is made that each drug acts independently of the other on the cells [27]. This is used to predict the combined response (C) for the two single compounds (A and B) by the formula: $C = A + B – A * B$. [28].

Results

MCS170 as a model to study mesenchymal chondrosarcoma behavior in vitro

MCS170 cells morphologically show an elongated shape, and they predominantly grow in groups of cells (Figure 1A). Using FISH we confirmed the presence of the *HEY1-NCOA2* intra-chromosomal inversion in all cells similar to the positive control (Figure 1B), colocalization of one set of red and green probes was observed while the other set of red and green probes were at limited distance. Expression of the HEY1-NCOA2 fusion product was confirmed using RT-PCR and Sanger sequencing revealed that exon 4 of

HEY1 was fused to exon 13 of *NCOA2* (Figure 1C). The expression of the HEY1-NCOA2 fusion product was variable between different passage numbers of the cell line. No mutations were detected in any of the 50 oncogenes and tumor suppressor genes, including *TP53*, *IDH1* and *IDH2,* in the Ion AmpliSeq™Cancer Hotspot Panel v2. Cell identity as determined by the Cell ID GenePrint 10 system (Promega) is available on request.

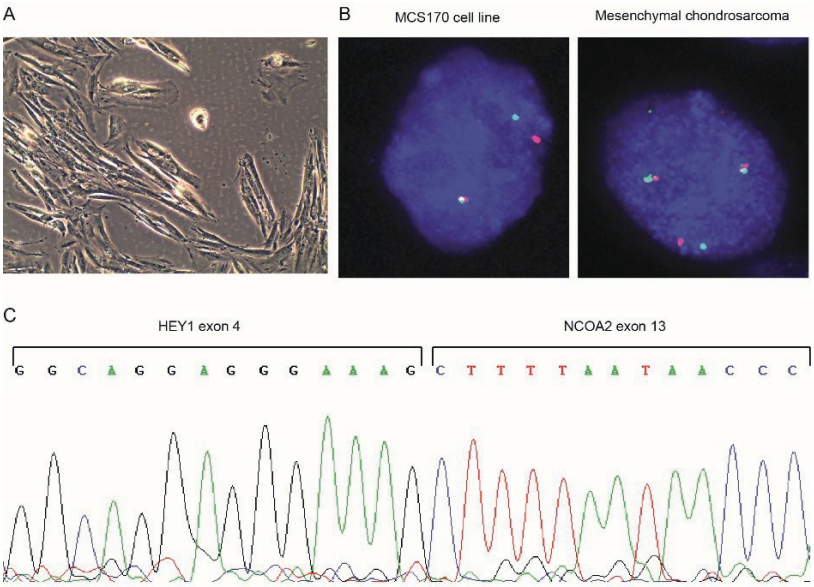

Figure 1: Characteristics of the MCS170 cell line.
A) Morphology of MCS170 cells in culture demonstrated elongated cytoplasm. Cells are growing predominantly in groups. Picture was taken at passage 31 with a 10 times objective. B) Interphase FISH using BAC clones RP11-152C15 and RP11-888F10 on the MCS170 cells and a Mesenchymal chondrosarcoma positive control sample C) Sequence showing the fusion of exon 4 of *HEY1* to exon 13 of *NCOA2* in MCS170 cells.

MCS170 cells are sensitive for doxorubicin, while resistant for cisplatin

To assess chemosensitivity, we treated MCS170 cells with the conventional chemotherapeutics doxorubicin or cisplatin. Cells were sensitive to doxorubicin treatment, especially after longer incubation periods, with an IC_{50} of 4.5 µM after 24h of treatment which decreased to an IC_{50} of 0.08 µM after 72h of exposure (Figure 2A and Table 1). Cells were resistant to cisplatin with an IC_{50} of 60 µM after 24 hours and 4.5 µM after 72 hours (Figure 2B).

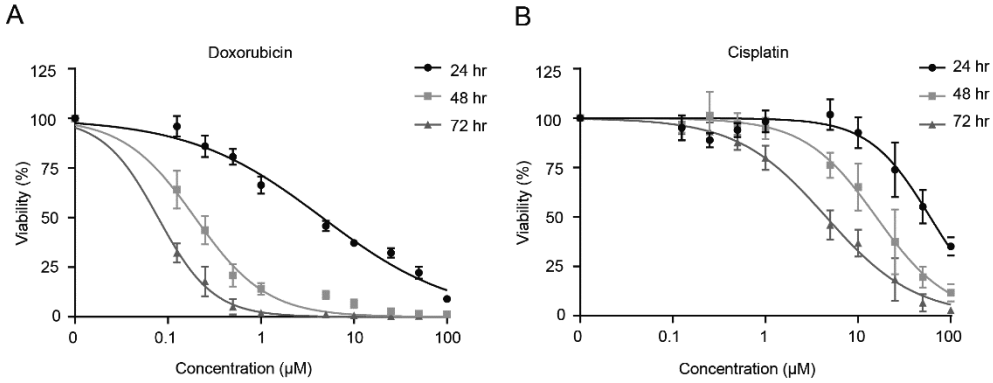

Figure 2: Sensitivity of MCS170 cells to doxorubicin and cisplatin
A, B) Dose response curves of MCS170 cells exposed to doxorubicin, cisplatin and ABT-737 at 24, 48 or 72 hours. Cells are sensitive for doxorubicin, especially when incubated for 72 hours (A), while more resistant for cisplatin (B)

Table 1. IC_{50} values of MCS170 cells treated with doxorubicin, cisplatin and ABT-737 for 24, 48 and 72 hours of exposure.

	IC_{50} 24 h	**IC_{50} 48 h**	**IC_{50} 72 h**
Doxorubicin	4.5 μM	0.2 μM	0.08 μM
Cisplatin	60 μM	15.8 μM	4.5 μM
ABT-737	5.5 μM	2.3 μM	1.8 μM

Bcl-2 family members are not essential for survival of MCS170 cells

Since previous studies indicated that high expression of Bcl-2 and Bcl-xL is a hallmark of mesenchymal chondrosarcoma [23], we determined the protein expression of Bcl-2 family members in the MCS170 cell line. Bcl-2 was expressed in MCS170 (Figure 3A) cells although variable staining intensities were observed between different cells. Bcl-xl was highly expressed in MCS170 cells (Figure 3C), however only very weak Bcl-w expression was observed (Figure 3E). To assess whether these Bcl-2 family members are essential for mesenchymal chondrosarcoma cells, these were inhibited in MCS170 cells using the BH3 mimetic ABT-737. After 72 hours of treatment an IC_{50} of 1.8 μM was observed (Figure 3G), showing that cells are not sensitive for Bcl-2 family member inhibition.

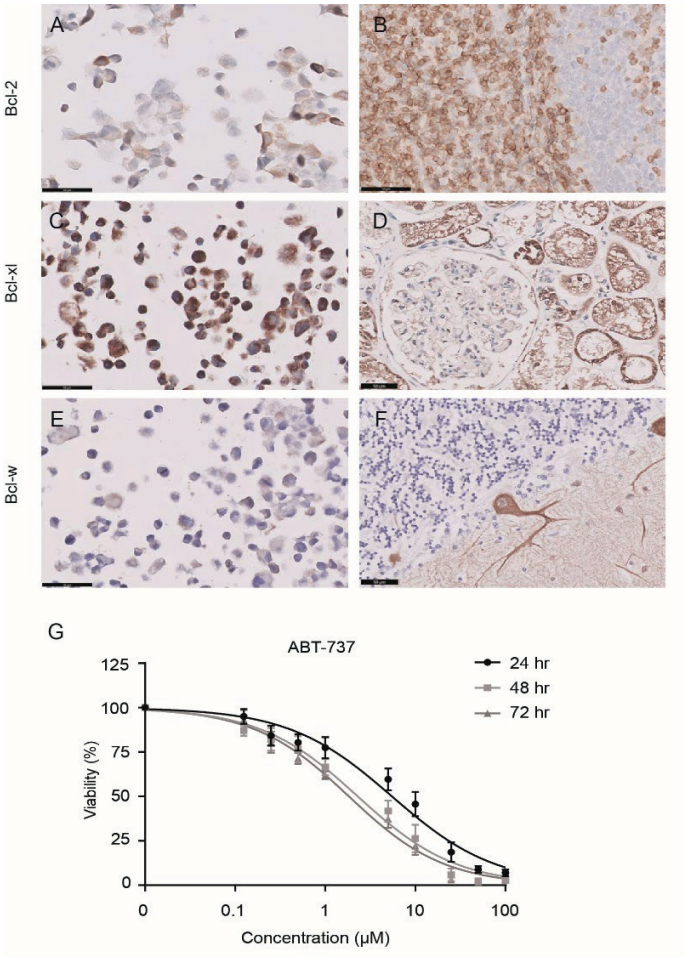

Figure 3: Bcl-2 and Bcl-xl are expressed in mesenchymal chondrosarcoma
A-F) Expression of Bcl-2(A, B) and Bcl-xl (C,D), but only low expression of Bcl-w (E,F) was found in paraffin embedded MCS170 cells (A, C, E), compared to expression in control tissues tonsil (B), kidney (D) and cerebellum (F) G) MCS170 cells show little sensitivity towards ABT-737 treatment after 24, 48 or 72 hours of exposure.

Inhibition of Bcl-2 family members sensitizes towards conventional chemotherapy

To evaluate a functional role for Bcl-2 family members in the response to chemotherapy MCS170 cells were incubated for 72 hours with ABT-737 and either doxorubicin or cisplatin. Compared to single treatment, a reduction in viability was observed when ABT-737 was combined with doxorubicin or cisplatin for 72 hours. (Figure 4A, B). To assess synergy the excess over Bliss

score was calculated. An excess over Bliss score of more than 12% (indicating synergy) was observed when 1 µM ABT-737 was used in combination with 125 nM doxorubicin or when 0.5 or 1 µM ABT-737 was combined with 5 µM cisplatin pointing towards a small synergistic effect (Table 2). Real time measurement of proliferation using the xCELLigence real time cell proliferation assay demonstrated an early significant reduction in cell index when 1 µM ABT-737 was combined with 1 µM doxorubicin, as compared to single treatment. Consistent with the viability assay, combining 1 µM ABT-737 with 125 nM doxorubicin or 5 µM cisplatin resulted in a reduction in cell viability at 72 hours of incubation and persisted until 96 hours (Figure 4C, D).

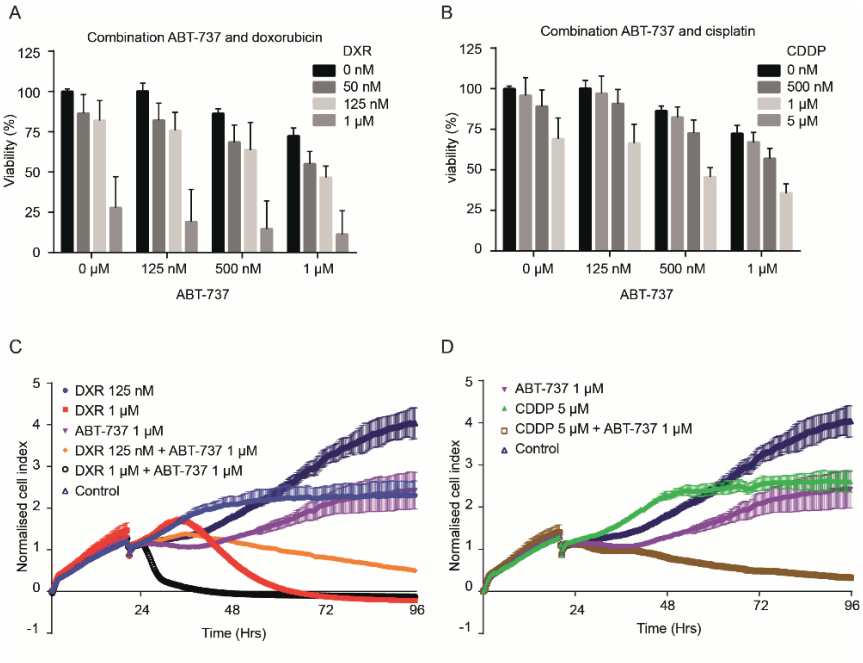

Figure 4: Combination treatment of ABT-737 and doxorubicin or cisplatin in MCS170 cells.
A,B) Viability after treatment of MCS170 cells for 72h with ABT-737 and doxorubicin (A) or ABT-737 and cisplatin (B). Combination treatment shows a larger reduction in viability compared to single treatment after 72 hours. C, D) Real time proliferation assay with the xCELLigence system of MCS170 cells treated with ABT-737 and doxorubicin (C) or ABT-737 and cisplatin (D). The combination of 1 µM ABT-737 and 1 µM doxorubicin is most effective and shows a faster reduction in cell index, compared to single treatment. The combinations of 1 µM ABT-737 and 125 nM or 5 µM cisplatin show a similar reduction in cell index and are more effective compared to single treatments.

Table 2. Excess over Bliss calculations of MCS170 cells treated with combinations of ABT-737, doxorubicin or cisplatin showing highest percentages when 1 μM ABT-737 was used in combination with 125 nM doxorubicin or when 1 or 0.5 μM ABT-737 is combined with 5 μM cisplatin.

Excess over Bliss (%)		**ABT-737**		
		1 μM	0.5 μM	0.125 μM
Doxorubicin	1 μM	8.9	9.4	8.8
	0.125 μM	12.6	7.0	6.3
	0.05 μM	7.5	6.1	4.4
Cisplatin	5 μM	14.4	14.1	2.8
	1 μM	7.6	4.4	-1.5
	0.5 μM	-5.5	-8.9	-12

Combination of ABT-737 with doxorubicin or cisplatin increases apoptosis

To assess if treatment with ABT-737 and combination with chemotherapy resulted in apoptosis, caspase 3/7 activity was measured after 24h of treatment (Figure 5A). Combination treatment of ABT-737 with doxorubicin or cisplatin for 24 hours substantially increased caspase 3/7 dependent apoptosis. This effect was inhibited by the pan caspase inhibitor Z-vad-FMK, confirming that the effect was caspase dependent (Figure 5A). Consistent with results obtained using xCELLigence, combining 1 μM ABT-737 with 1 μM doxorubicin resulted in a large reduction of cell viability, after 24 hours (Figure 5B). However this effect could not completely be restored by adding z-vad, indicating that additional mechanisms that reduce viability, besides caspase dependent apoptosis, may be operable. PARP cleavage was also measured using western blot (Figure 5C), confirming that combination treatment results in activation of apoptosis.

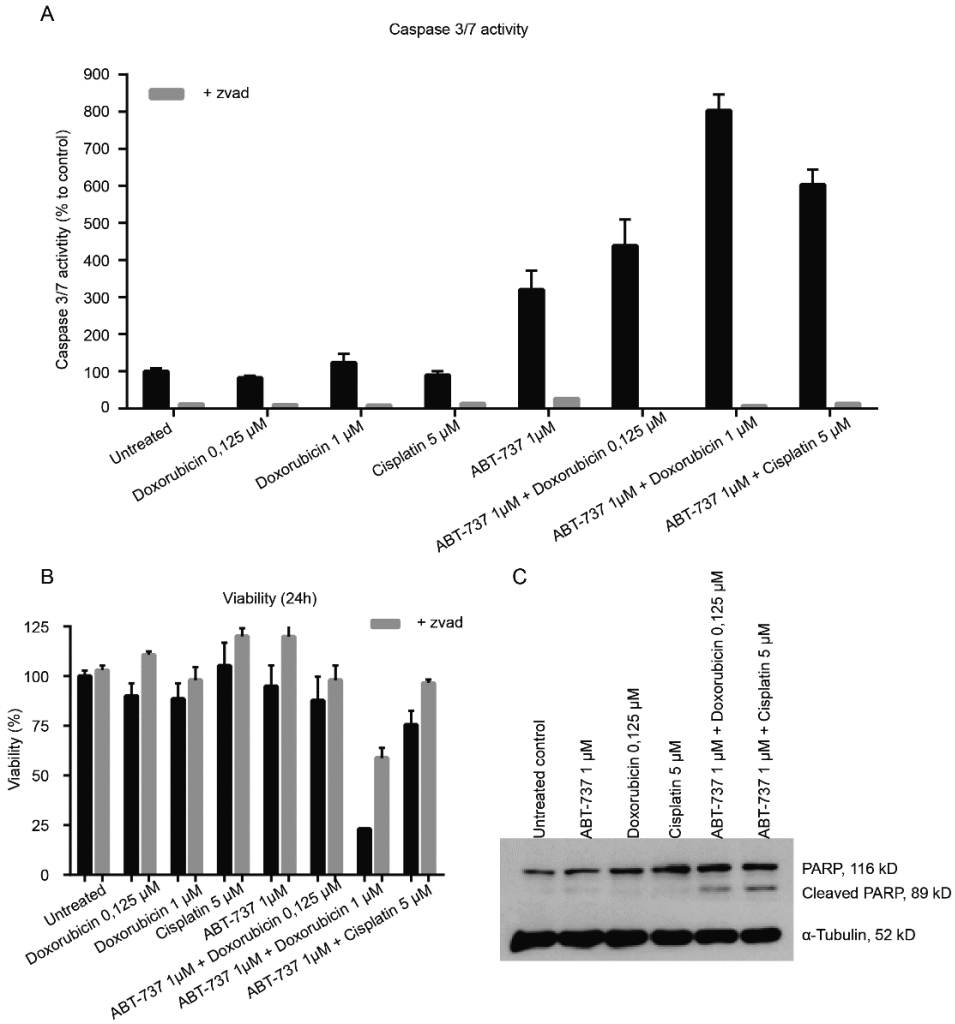

Figure 5: Apoptosis activation after combining ABT-737 and doxorubicin or cisplatin in MCS170 cells.
A, B) Caspase 3/7 activity (A) and viability (B) measured after 24 hours of treatment with doxorubicin, cisplatin or ABT-737 and the combination showing highest caspase 3/7 activity and lowest viability in MCS170 cells treated with 1 µM ABT-737 combined with 1 µM doxorubicin. Pan-caspase inhibitor Z-vad-FMK inhibits all the caspase 3/7 activity and restores part of the viability reduction observed after treatment with 1 µM ABT-737 and 1 µM doxorubicin. C) Western blot image showing PARP cleavage in cells treated with 1 µM ABT-737 and 125 nM doxorubicin or 5 µM cisplatin.

Discussion

We here present the first mesenchymal chondrosarcoma cell line; MCS170, in which we could demonstrate that inhibition of Bcl-2 family members, can restore chemosensitivity. Mesenchymal chondrosarcoma is an aggressive chondrosarcoma subtype, with a poor prognosis. There is an urgent need for alternative treatment strategies, as the current prognosis is very poor with reported 10 years survival rates between 27% and 67% [6-9].

The generation of the MCS170 cell line as a model for mesenchymal chondrosarcoma will give the opportunity to perform more preclinical research to identify possible therapeutic candidates to treat patients with mesenchymal chondrosarcoma, as well as to study the functional role of the HEY1-NCOA2 fusion. The presence of the HEY1-NCOA2 fusion gene was confirmed at the DNA level and at the RNA level. As expected in translocation driven sarcomas, a low mutational burden was found, as no additional mutations were found in 50 oncogenes or tumour suppressor genes frequently mutated in cancer.

Preclinical studies on mesenchymal chondrosarcoma are rare, and only few possible therapeutic options have been described based on expression analysis in small sample groups. A small study investigating a panel of different proteins in three mesenchymal chondrosarcoma cases identified PKC-α, PDGFR, Bcl-2 [29] and mTOR [30] as potential therapeutic targets. In a larger study including 23 cases of mesenchymal chondrosarcoma TGF-β, Bcl-2 and Bcl-xL were identified as possible therapeutic targets in mesenchymal chondrosarcoma [23]. No mutations in hot spot regions of *TP53* were identified in a panel of 33 mesenchymal chondrosarcomas; however 61.3% showed nuclear overexpression of the p53 protein [31].

In this study we focused on evaluating the role of Bcl-2 family members and conventional chemotherapy in mesenchymal chondrosarcoma. The anti-apoptotic proteins Bcl-2 and Bcl-xL were found to be highly expressed in chondrosarcoma, especially in the mesenchymal subtype, suggesting an important role for these proteins in mesenchymal chondrosarcoma chemoresistance and thereby a possible novel treatment strategy [23].

The MCS170 cell line was sensitive for doxorubicin, especially when cells were treated for 72 hours, but resistant for cisplatin. This is in line with a recently published retrospective study including 119 patients, showing that mesenchymal chondrosarcoma patients with localized disease have reduced risk of recurrence when treated with anthracycline based chemotherapy

suggesting that it is beneficial to add adjuvant chemotherapy to the standard treatment regimen [7]. However, a recent analysis of the available literature could not confirm these data [19]. Also the study of Xu et al. did not detect a difference in overall survival when patients with localised disease were compared with patients that showed metastasis.

Bcl-2 and especially Bcl-xL were found to be highly expressed in the MCS170 cell line, which is consistent with the known high expression of these Bcl-2 family members in mesenchymal chondrosarcoma primary tumours [23]. To investigate the role of Bcl-2 family members in mesenchymal chondrosarcoma, the BH3 mimetic ABT-737 was used to inhibit the anti-apoptotic proteins Bcl-2, Bcl-xL and Bcl-w in MCS170. While ABT-737 monotherapy was ineffective, combination of ABT-737 and doxorubicin resulted in a synergistic induction of apoptosis, and reduction in viability, suggesting a role for Bcl-2 family members in resistance to chemotherapy in mesenchymal chondrosarcoma.

Induction of apoptosis is important for the effectiveness of many therapies, which can be prevented by high levels of anti-apoptotic proteins or down regulation of pro-apoptotic proteins. By directly targeting the intrinsic apoptotic pathway using BH3 mimetic ABT-737, more pro-apoptotic proteins will be available to activate Bak and Bax, resulting in higher efficacy of other therapies that induce apoptosis [24].

The combination of Bcl-2 family protein inhibitors and chemotherapeutics has activity in vitro in a wide variety of tumours [32], including conventional as well as dedifferentiated chondrosarcoma [21, 23]. Now that the MCS170 cell line is available, we had the opportunity to show this experimentally in a mesenchymal chondrosarcoma model as well, indicating that patients with mesenchymal chondrosarcoma might also benefit from this treatment combination.

ABT-263 (Navitoclax) is an orally available derivative of ABT-737 with the same working mechanism, which makes it a more attractive compound compared to ABT-737. ABT-263 is currently under evaluation in clinical trials, however due to side effects caused by the high Bcl-xL expression on platelets, only low doses could be administered [33]. Here we demonstrate that treatment with BH3 mimetics can sensitize mesenchymal chondrosarcoma for conventional chemotherapy, while single agent administration is not effective. This raises the possibility to administer these agents in combination, increasing the activity, while lowering toxicity in mesenchymal chondrosarcoma.

Mesenchymal chondrosarcoma is a rare aggressive chondrosarcoma subtype with a poor prognosis. In this study we describe the first mesenchymal chondrosarcoma cell line; MCS170, containing the characteristic *HEY1-NCOA2* fusion, that can be used as a model to identify new therapeutic targets. As mesenchymal chondrosarcoma is known to have high Bcl-2 and Bcl-xl expression [23], we used this cell line to functionally investigate the role of Bcl-2 family members in chemosensitivity. We demonstrate that inhibition of Bcl-2 family members restores the apoptotic machinery in mesenchymal chondrosarcoma, rendering the cells sensitive to conventional chemotherapy, especially doxorubicin.

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