ELSEVIER

Journal of Hepatology 44 (2006) 151–157

Journal of Hepatology

www.elsevier.com/locate/jhep

Mcl-1 overexpression in hepatocellular carcinoma: A potential target for antisense therapy

Wolfgang Sieghart[1,2], Doris Losert[1], Sabine Strommer[1], Daniel Cejka[1], Katharina Schmid[3], Susanne Rasoul-Rockenschaub[4], Martin Bodingbauer[4], Richard Crevenna[5], Brett P. Monia[6], Markus Peck-Radosavljevic[2], Volker Wacheck[1,*]

[1]*Section of Experimental Oncology/Molecular Pharmacology, Department of Clinical Pharmacology, Medical University Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria*

[2]*Division of Gastroenterology/Hepatology, Department of Internal Medicine IV, Medical University Vienna, Vienna, Austria*

[3]*Department of Clinical Pathology, Medical University Vienna, Vienna, Austria*

[4]*Division of Transplantation Surgery, Department of Surgery, Medical University Vienna, Vienna, Austria*

[5]*Department of Physical Medicine and Rehabilitation, Medical University Vienna, Vienna, Austria*

[6]*ISIS Pharmaceuticals Inc, Carlsbad, California, USA*

***Background/Aims*: Recently, the anti-apoptotic Mcl-1 protein has been reported as a resistance factor in various types of cancer. Here we investigated the presence of Mcl-1 protein in hepatocellular carcinoma (HCC) tissues and its potential role as a molecular drug target for HCC therapy.**

***Methods*: HCC specimens of 149 patients were examined by immunohistochemistry for Mcl-1 expression. Antisense oligonucleotides (ASO) targeting Mcl-1 were evaluated as monotherapy and in combination with cisplatin in the HCC cell lines HepG2 and Snu398. Protein regulation, cell viability, and apoptosis were assessed by western blotting, cell counting, and FACS analysis.**

***Results*: Mcl-1 protein is overexpressed in 51% of all cases irrespective of underlying disease. Targeting Mcl-1 by ASO specifically downregulated Mcl-1 protein expression and led to significant dose and time dependent single agent activity in HCC cells characterized by increased apoptosis and decreased cell viability. No significant target regulation or cell death was observed for control oligonucleotide treatment. Upon combination with cisplatin, Mcl-1 ASO revealed a significant chemosensitizing effect.**

***Conclusions*: Mcl-1 is overexpressed in half of HCC-tissues. ASO targeting Mcl-1 revealed a prominent single agent and chemosensitizing activity against HCC in vitro. Targeting Mcl-1 might qualify as a promising novel approach in HCC therapy.**

***Keywords*: HCC; Mcl-1; Targeted therapy; Antisense oligonucleotide**

1. Background

Hepatocellular carcinoma (HCC) is the 5th most common cancer world wide and 564,000 new cases are diagnosed every year. At advanced stage of disease, the overall outcome for most patients is poor with few treatment options available [1].

However, the progress of basic cancer research and oncogenomics over the last decade uncovered several potential target proteins for developing rational and more effective anticancer agents for novel molecular targeting therapeutic strategies.

One of these potential target proteins is the antiapoptotic Mcl-1 protein. Mcl-1 is a member of the Bcl-2 protein

Received 19 May 2005; received in revised form 25 August 2005; accepted 9 September 2005; available online 25 October 2005

* Corresponding author. Tel.: +43 1 40400 2981; fax: +43 1 40400 2989.

E-mail address: volker.wacheck@meduniwien.ac.at (V. Wacheck).

0168-8278/$30.00 © 2005 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jhep.2005.09.010

family and is expressed in various tissues and malignant cells [2–4]. Mcl-1 contains the Bcl-2 homology domains BH 1–3 [5] and has been shown to be involved in apoptosis and cell cycle [6]. In contrast to Bcl-2, Mcl-1 is rapidly inducible [7,8], and has a short half life depending on the cell type considered [2,9]. In addition, Mcl-1 seems to be more widely distributed within the cell than Bcl-2: besides its predominant localization at the mitochondrial membranes, Mcl-1 has also been found in the nucleus and the cytoplasm [2,10].

Several mechanisms of action have been suggested for the antiapoptotic action of Mcl-1. It has been proposed that Mcl-1 might deploy its antiapototic potential by avoiding cell damage-induced mitochondrial cytochrome C release [11]. Moreover, Mcl-1 is able to heterodimerize with Bax neutralizing its proapoptotic activitiy, if cells are exposed to apoptotic stress [7,12].

Even though the definite mechanisms of how Mcl-1 promotes cell survival is not yet fully elucidated, the biological significance of Mcl-1 protein expression in supporting cell survival has been well documented in a number of cell systems including human myeloblastic leukaemia [13] and myeloma [14] cells. Furthermore, Mcl-1 is overexpressed in many human tumor specimens [15–18], including metastasis from colorectal cancer to the liver, where diffuse expression indicates a treatment resistant phenotype [19].

To date, no data are available about the expression of Mcl-1 in primary hepatocellular carcinoma.

A therapeutic strategy for modulating selectively the expression of a gene of interest is the use of antisense oligonucleotides (ASO). ASO are chemically modified stretches of single stranded DNA binding to their complementary target mRNA leading to selective inhibition of target protein expression [20,21]. Antisense strategies targeting Bcl-2, Bcl-xL and Mcl-1 using specific ASOs have displayed promising results in a various human malignancies in vitro and in vivo [22–26].

In this study we investigated, (1) the prevalence of Mcl-1 protein expression in human HCC samples and (2) its biological significance for proliferation and chemoresistance by using an antisense oligonucleotide (ASO) strategy.

2. Materials and methods

2.1. Patient samples

Hepatocellular carcinoma tissues from 149 patients (21 females and 128 males) undergoing orthotopic liver transplantation (OLT) were analyzed. The average age of patients was 55 ± 9 (SD) years and the mean tumor size was 5.3 ± 4.8 (SD) cm at the time of OLT. Information about underlying liver diseases was available and is given in Table 1. Fifty-one patients received treatment before OLT including chemotherapy with doxorubicin ($n=22$), percutaneous ethanol instillation ($n=10$), radiofrequency ablation ($n=7$), chemoembolisation ($n=2$) or surgical resection ($n=10$).

2.2. Immunohistochemistry

A tissue array consisting of three representative 0.6 mm cores from paraffin embedded tissue blocks from each of the 149 hepatocellular carcinomas was constructed.

Sections were dried at 55 °C and then deparaffinized in xylene for 10 min, followed by dehydration through graded alcohols. Afterwards tissue proteolysis was performed by pre-treatment using an autoclave. Subsequently sections were immersed in PBS-buffered saline (pH 7.6) and incubated with a Mcl-1 antibody (Labvision, Fremont, CA, USA) at a dilution of 1:200 or Ki67 antibody (Novocastra, Newcastle, UK) at a dilution of 1:20 for 1 h at room temperature. Negative controls were treated with normal goat serum without primary antibodies and with appropriate isotype control. The detection of antibodies was performed with a Labvision immunohistochemistry kit.

For the evaluation of the staining the whole area of the section was used. Samples with immunostaining of less than 10% of the tumor cells were considered to be negative. Samples with 11–40%, 41–70% or >71% positive tumor cells were scored as weakly (+), moderate (++) and strongly (+++) positive, respectively.

2.3. Cell culture

The hepatocellular carcinoma cell lines HepG2 and Snu398 were obtained from American Type Culture Collection (ATCC, Manassas, VA). Both cell lines were maintained in basal tissue culture medium (RPMI, Gibco BRL, Paisley, Scotland) supplemented with 10% fetal calf serum (FCS) and 1% antibiotic–antimycotic mix (Gibco BRL, Paisley, UK), in a humidified 5% CO_2, 95% ambient air atmosphere at 37 °C.

2.4. Oligonucleotides

2′-O-Methoxyethyl/2′-deoxynucleotide chimeric phosphorothioate ASOs were kindly provided by ISIS Pharmaceuticals (Carlsbad, CA). The sequence of the Mcl-1 ASO (ISIS 20408) was 5′-TTGGCTTTGTGTCCTTGGCG-3′. The universal control oligonucleotide (UC) used in this study was synthesized as a mixture of A, G, T, and C bases so that the resulting preparation contained an equimolar mixture of all

Table 1
Mcl-1 expression in HCC with respect to underlying liver disease

Underlying liver disease	Total *N*	Mc1-1 expression			
		Negative *N*	+*N*	++*N*	+++*N*
Alcoholic cirrhosis	46 (31%)	22 (48%)	14 (30%)	7(15%)	3(7%)
Hepatitis C cirrhosis	43 (29%)	21 (49%)	14 (33%)	6 (14%)	2 (5%)
Non cirrhotic Liver	19 (13%)	10 (53%)	6 (32%)	2 (11%)	1 (5%)
Hepatitis B cirrhosis	14 (9%)	8 (57%)	5 (36%)	0	1 (7%)
Cryptogenic cirrhosis	9 (6%)	2 (22%)	3 (33%)	3 (33%)	1 (11%)
Other cirrhosis	18 (12%)	10 (56%)	4 (22%)	3 (17%)	1 (6%)

The term ‘other cirrhosis’ includes haemochromatosis ($n=3$), primary biliary cirrhosis ($n=3$), primary sclerosing cholangitis ($n=1$), cirrhosis due to hepatitis BC ($n=2$) and BCD ($n=1$) coinfection, post-hepatitic cirrhosis ($n=1$) and cirrhosis of other viral origin ($n=7$).

possible 4 to the 19th nucleotides. The oligonucleotide backbone chemistry of UC is identical to that of ASO.

2.5. Treatment of cells and assessment of cell growth

HepG2 and Snu398 cells were seeded at density of 15.000 cells per cm^2. For transfection, cells were seeded 24 h before the oligonucleotide treatment. Oligonucleotides were complexed with Lipofectin (Life Technologies, Paisley, United Kingdom) at a ratio of 1:3 in antibiotic-free medium without serum as described by the supplier. Subsequently, cells were incubated with different concentrations of complexed oligonucleotides in antibiotic-free medium without serum. After 5 h of incubation, transfection was stopped with standard medium. Cell growth was determined by analysing cell numbers using a Coulter Z1 counter (Coulter, Luton, Beds., UK).

For combination treatment with chemotherapy, HepG2 and Snu398 cells were incubated with cisplatin (Ebewe, Unterach, Austria) 24 h after transfection with oligonucleotides at the concentrations as indicated.

2.6. Western blotting

For analysis of Mcl-1 protein expression cells were seeded and treated with oligonucleotides after 24 h as described above.

Western blotting of lysed cells for Mcl-1 and Tubulin was performed as previously described [22]. In brief, whole cell extracts were prepared using 0.14 M NaCl, 0.2 M Triethanolamine, 0.2% Na-deoxycholate, and 0.5% Nonidet P-40, supplemented with protease inhibitors (all Sigma, St Louis, MO, USA). 10 μg of total protein were separated by SDS-PAGE and blotted to PVDF membranes (Tropix, Bedford, MA, USA). The membranes were blocked for 1 h in 0.2% I-block (Tropix) in PBS, and then incubated with monoclonal antibodies binding to Mcl-1 (Santa Cruz Biotechnology, Santa Cruz, CA) or β-Tubulin (Sigma, St Louis, MO, USA), respectively. Second antibody incubations were carried out using goat anti-rabbit or goat anti-mouse conjugated to alkaline phosphatase (Tropix). Reactive bands were detected by chemiluminescence (CSPD substrate, Tropix). Equal protein loading in each lane was documented by detecting Tubulin protein expression. The expression levels of Mcl-1 and Tubulin protein were quantified by densitometry (TotalLab, version 1.1, UK). Intensity of each Mcl-1 signal was normalized against the respective Tubulin control. Changes in protein expression less than 20% were regarded as not significant.

2.7. Assessment of apoptosis

Apoptotic cells were identified by their sub-diploid DNA content using flow cytometrical analysis as previously described [27]. Cells were washed in PBS, fixed in ice cold 70% ethanol for 1 h, washed in PBS and incubated in PBS containing 0.1% DNase-free RNase A and 100 μg/mL propidium iodide for 30 min. A minimum of 1.5×10^4 events was analyzed on a FACScalibur flow cytometer with an argon laser tuned at 488 nm and use of the CellQuest software (both Becton Dickinson, New Jersey, USA). Hypochromatic cells due to chromatin condensation with sub-diploid DNA profiles were identified as apoptotic fraction of cells. Cell debris was excluded from analysis by appropriate light scatter gating.

2.8. Statistical analysis

Unless otherwise stated, all data are expressed as means relative to saline control ± standard error of the mean (SEM) and derived from at least three independent experiments. Statistical significance of differences among treatment groups was calculated by using one-way ANOVA and Bonferroni's test was used for post-hoc comparisons using SPSS software (SPSS 10.0.7, SPSS Inc., Chicago, IL). *P*-values less than 0.05 were considered to be of statistical significance.

3. Results

3.1. Mcl-1 is expressed in HCC tissue samples

To evaluate the presence of Mcl-1 protein expression in HCC, we analyzed tumor specimens of 149 patients by

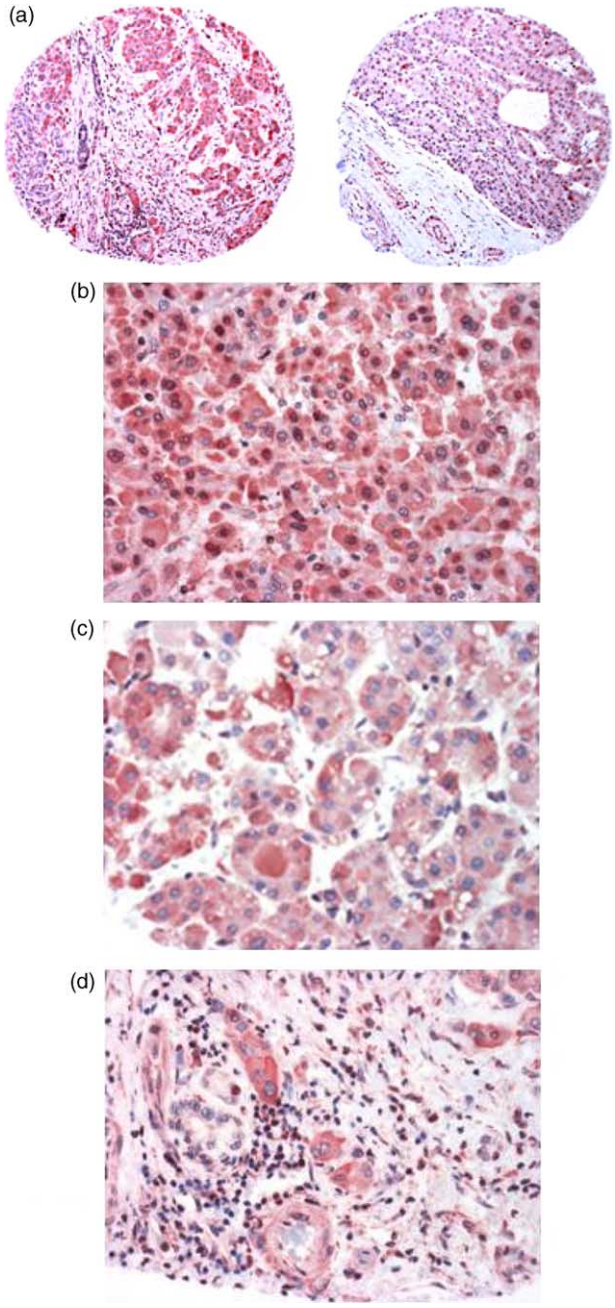

Fig. 1. (a–d): Mcl-1 is expressed in HCC tissue specimens. Four representative images of Mcl-1 staining in hepatocellular carcinoma with original magnification ×100 for (a) and ×400 for (b–d): (a) on the left: a whole tissue array spot with (++) staining of Mcl-1 protein; on the right: a whole tissue array spot with no significant staining of Mcl-1 protein (b) (+++) staining of Mcl-1 protein (c) (+) staining of Mcl-1 protein (d) isolated invasive tumor cells with positive Mcl-1 staining in adjacent fibrotic tissue. [This figure appears in colour on the web.]

immunohistochemistry. In total, 76 of 149 patients (51%) were positive for Mcl-1 protein expression (Table 1). The Mcl-1 staining pattern in tumor cells was primarly cytoplasmatic and partially intranuclear (Fig. 1(a)–(d)). There was no correlation between Mcl-1 expression and tumor size, *tumor grade* or Ki-67 expression (Spearman Rank Test n.s.). No significant differences in Mcl-1 expression were observed with regard to underlying liver disease or treatment before liver transplantation. In paired samples of non-tumor liver tissue adjacent to HCC (21 cases) no positive staining was observed besides isolated weak positivity in bile ducts and cirrhotic nodules, which were directly neighbouring tumor tissue.

To test whether Mcl-1 is overexpressed in cases where other anti-apoptotic proteins are not, we additionally stained survivin and Bcl-XL in the available tissue array. We found a significant correlation between Mcl-1 and Bcl-XL expression (Spearman rank test: $N=149$, $r=0.3$, $p=0.0003$). No correlation was observed between Mcl-1 and survivin expression (Spearman rank test: $N=149$, $r=0.066$, $p=0.455$).

3.2. Antisense oligonucleotides downregulate Mcl-1 protein expression in HCC cell lines

Given the overexpression of Mcl-1 in HCC, we next evaluated the potency of ASO for selective targeting of Mcl-1 protein in hepatocellular carcinoma cells.

Mcl-1 ASO at a concentration (100 nM) shown to be effective in previous studies [23,25] specifically downregulated Mcl-1 protein expression in HepG2 and Snu398 cells beginning from 24 h after transfection (Fig. 2(a)).

Subsequent dose range experiments using 50–200 nM of ASO revealed a dose dependent downregulation of Mcl-1 protein levels (Fig. 2(b)). Whereas Mcl-1 level at 50 nM ASO were only slightly decreased by $29\pm7\%$ (mean $\pm$ SEM) not differing significantly from saline control ($P>0.05$), Mcl-1 ASO at 100 and 200 nM resulted in a similar clear silencing of Mcl-1 by $57\pm19\%$ and $60\pm5\%$ compared to saline treated control (SAL), respectively (for both $P<0.05$). At both concentrations of ASO, Mcl-1 levels differed significantly from UC oligonucleotide treated cells ($P<0.05$ for both concentrations). Thus, we considered 100 nM as the minimal effective dose for biological activity of Mcl-1 ASO and used this concentration for chemosensitisation experiments.

In order to determine persistence of Mcl-1 downregulation by Mcl-1 ASO, we performed additional time course experiments (Fig. 2(c)): 48 and 72 h after transfection there was still a significant reduction in Mcl-1 protein levels compared to saline treated control ($44\pm12\%$ and $43\pm6\%$; $P<0.005$ for both time points). No significant target regulation was observed for UC control treatment at all time points studied (UC vs. SAL: $P>0.05$) and UC control treatment differed significantly from ASO treatment ($P<0.005$).

3.3. Downregulation of Mcl-1 protein expression induces cell death in HCC cell lies

Since Mcl-1 is an antiapoptotic protein, we next evaluated the single agent activity of Mcl-1 ASO on cell growth and apoptosis of hepatocellular carcinoma cells by cell counting and FACS analysis. Time course experiments

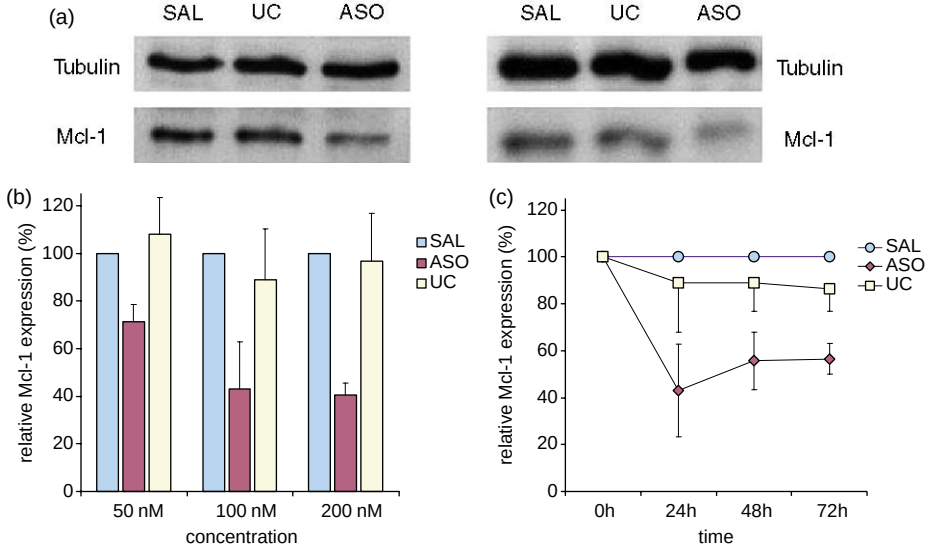

Fig. 2. (a–c): Dose- and time-dependent downregulation of Mcl-1 protein expression by Mcl-1 ASO. HCC cell lines were assessed for Mcl-1 expression by western blotting after treatment with Mcl-1 ASO, universal control oligonucleotides (UC) and saline (SAL) in the presence of the uptake enhancer lipofectin: (a) Mcl-1 expression of HepG2 (left) and Snu398 cells (right) 24 h after transfection with 100 nM ASO (b) dose-response of Mcl-1 expression using 50–200 nM of ASO 24 h after transfection of HepG2 cells (c) time course of Mcl-1 expression 24, 48 and 72 h after transfection of HepG2 cells with 100 nM of ASO. All data are expressed relative to saline control; Bars = SEM. [This figure appears in colour on the web.]

with Mcl-1 ASO single agent treatment (100 nM) revealed an increase of apoptosis by about 55% beginning from 48 h after transfection compared to saline control (mean ± SEM; 48 h: 53 ± 16%; 72 h: 58 ± 19%; $P < 0.001$ for both time points) (Fig. 3(a)). This increase of apoptosis was mirrored by a significant time dependent reduction of cell numbers by about 35% compared to saline treated control (48 h: 31 ± 10%; 72 h: 39 ± 8%; $P < 0.005$ for both time points) (Fig. 3(b)). Consistent with western blot results showing no target regulation, UC at the dose of 100 nM revealed no significant cytotoxicity assessing apoptosis or cell counts (UC vs. SAL: $P > 0.05$ at any time point) but differed significantly from Mcl-1 ASO treatment (ASO vs. UC: $P < 0.05$ at any timepoint) (Fig. 3(a) and (b)).

Mcl-1 ASO effects observed on apoptosis induction and reduced cell count were dose dependent (Fig. 3(c) and (d)). In line with Mcl-1 downregulation results, dose range experiments with 50–200 nM of ASO confirmed a concentration of 100 nM of Mcl-1 ASO to be the minimal effective dose to induce cell death in HCC cells whereas UC oligonucleotides at the same concentration did not differ significantly from saline control ($P > 0.05$).

3.4. Mcl-1 ASO sensitize HCC cells to cisplatin

It was recently demonstrated that downregulation of Mcl-1 sensitizes malignant melanoma and human sarcoma to apoptosis inducing chemotherapeutics [23,25]. Thus, we also evaluated the chemosensitizing potential of Mcl-1 ASO to cisplatin, an apoptosis inducing chemotherapeutic commonly used for treatment of HCC [28].

In pre-experiments we determined 0.1 μg/mL cisplatin as a concentration resulting in an about 20% reduction of cell numbers of HepG2 and Snu398 cells (data not shown). Combination of cisplatin (0.1 μg/mL) and Mcl-1 ASO at the minimal effective dose (100 nM) (Fig. 4(a) and (b)) doubled number of apoptotic cells compared to cisplatin alone and exceeded apoptosis rates of Mcl-1 ASO monotherapy by 50% (ASO + cisplatin vs. SAL + cisplatin: $P < 0.005$; ASO + cisplatin vs. ASO alone: $P < 0.01$).

This increase of apoptosis led to a concurring reduction of cell counts. Compared to cisplatin or Mcl-1 ASO single agent treatment, the combination of cisplatin and Mcl-1 ASO further reduced cell counts by 42 and 18% 72 h after transfection, respectively, (ASO + cisplatin vs. SAL + cisplatin: $P < 0.001$; ASO + cisplatin vs. ASO alone: $P =$

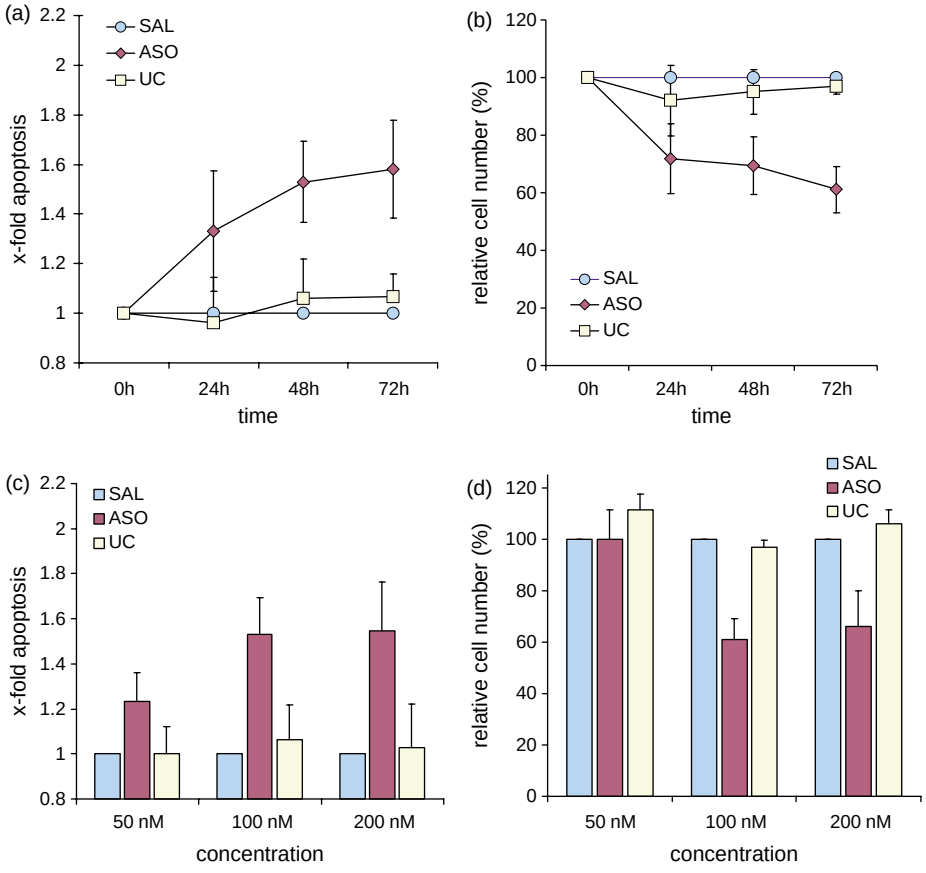

Fig. 3. (a–d): Single agent activity of Mcl-1 ASO. Induction of cell death by treatment with ASO targeting Mcl-1, universal control oligonucleotides (UC) and saline (SAL) in the presence of the uptake enhancer lipofectin in HepG2 cells: time course of apoptosis analyzed by FACS (a) and cell growth (b) at 24, 48 and 72 h after transfection of HepG2 cells with 100 nM Mcl-1 ASO. Dose-response of apoptosis analyzed by FACS (c) and cell growth (d) using 50–200 nM Mcl-1 ASO, 48 and 72 h after transfection, respectively. All data are expressed relative to saline control; Bars = SEM. [This figure appears in colour on the web.]

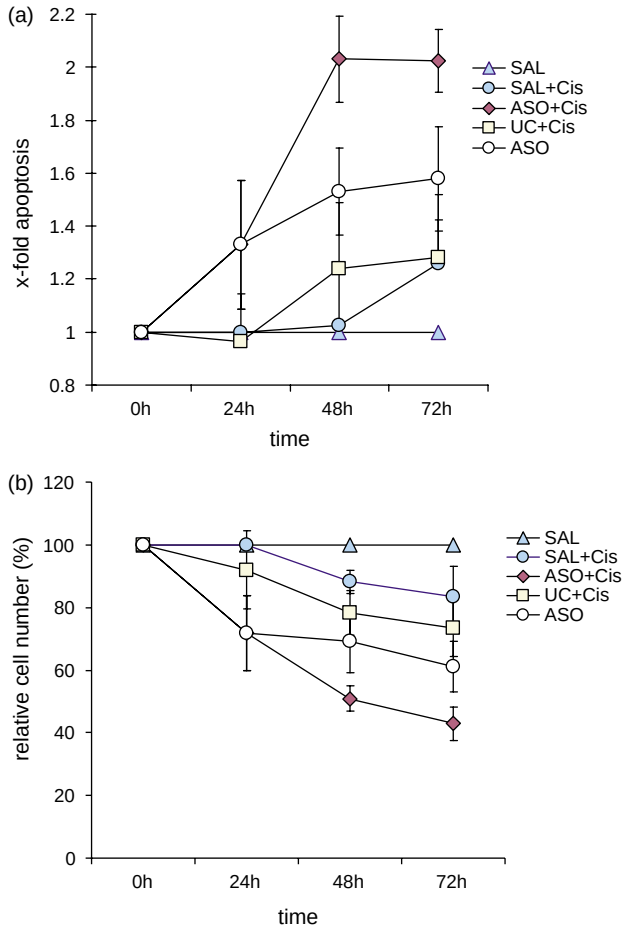

Fig. 4. (a–b): Mcl-1 ASO chemosensitize HCC to cisplatin. To assess combination treatment strategies, HepG2 cells were pre-treated for 5 h with Mcl-1 ASO, universal control oligonucleotides (UC) and saline (SAL) at a concentration of 100 nM in presence of the uptake enhancer lipofectin. Twenty-four hour after transfection, cells were incubated with low dose cisplatin (0.1 μg/mL) and evaluated for apoptosis by FACS analysis (a) and cell growth (b). All data are expressed relative to saline control; Bars = SEM. [This figure appears in colour on the web.]

0.01). There was no significant difference in apoptosis or cell counts between UC + cisplatin and SAL + cisplatin ($P > 0.05$ for apoptosis and cell counts at all time points), supporting a specific apoptosis inducing effect of Mcl-1 ASO. Similar results were observed with Snu398 cells.

4. Discussion

To the best of our knowledge, this is the first study investigating Mcl-1 expression in human HCC tissue. In a screen of 149 HCC tissue specimens Mcl-1 was overexpressed in 51% of all cases, correlated positively with Bcl-XL expression, but was not influenced by underlying liver disease. Notably, no significant Mcl-1 protein expression was observed in adjacent liver tissue in this study, which is in line with previous results [29]. Thus, our findings highlight Mcl-1 overexpression as a cancer specific event. Since liver tissue specific deletion of Mcl-1 in vivo does not induce apoptosis of normal hepatocytes [30], targeting Mcl-1 for HCC therapy might not harm healthy liver tissue.

Therefore, we performed in vitro studies to investigate the biological significance of Mcl-1 in HCC cell lines. Low nanomolar concentrations of ASO targeting Mcl-1 resulted in a profound, long-lived downregulation of Mcl-1 protein expression in a dose dependent manner. Notably, and in contrast to previous studies in solid tumors [23,25], targeting Mcl-1 in HCC resulted in spontaneous apoptosis without an additional apoptotic stimulus. This single agent anti-tumor activity of Mcl-1 ASO is most likely related to the specific downregulation of Mcl-1 protein expression because of the following reasons: first, neither lipofectin control treatment (saline control) nor universal control oligonucleotides with the identical backbone chemistry as the ASO induced apoptosis in HCC cells. Secondly, the applied ASO compounds do not affect protein levels of Bcl-2 or Bcl-xL, two other antiapoptotic members of the Bcl-2 family with homology to the Mcl-1 mRNA [25]. Furthermore, also Bax protein levels remained unchanged during transfection with Mcl-1 ASO (data not shown) indicating that downregulation of Mcl-1 in HCC cell lines is sufficient to cause apoptosis induction by an unilateral shift of the sensitive balance between pro-and antiapototic proteins, even though the exact antiapoptotic mechanism of Mcl-1 is not known.

Finally, downregulation of Mcl-1 sensitize HCC cells to low dose cisplatin. This finding might become clinically relevant, since cisplatin is widely administered locally and systemically in the treatment of advanced HCC [28]. Unfortunately, response rates are not ideal and strategies are urgently needed to overcome chemoresistance of this malignant disease. Antisense oligonucleotides have already entered phase III clinical trials for modulation of chemoresistance in a variety of human malignancies [31]. One of the main issues for clinical use of ASO for cancer therapy is their adequate delivery to peripheral tumor sites, as ASOs get rapidly pooled in the liver after systemic application [32]. Indeed, this pharmacokinetic property of ASOs might qualify them particularly for treatment of HCC ensuring effective delivery to the tumor site in the liver.

In conclusion, Mcl-1 is overexpressed in half of HCC's. Specific downregulation of Mcl-1 in HCC cells leads to significant apoptosis induction and chemosensitization to cisplatin. Thus, Mcl-1 appears to be an attractive molecular target in HCC. Given the hepatotropic pharmacokinetic properties and low toxicity of Mcl-1 ASO in vivo [23,25] targeting Mcl-1 by ASO might qualify as a promising novel approach in HCC therapy warranting further in vivo studies.

Acknowledgements

This study was supported by the 'Medizinisch-wissenschaftlichen Fonds des Buergermeisters der Bundeshauptstadt Wien'. We thank Thorsten Fuereder and Fritz Wrba for their valuable support.

References

[1] Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003;362:1907–1917.
[2] Yang T, Kozopas KM, Craig RW. The intracellular distribution and pattern of expression of Mcl-1 overlap with, but are not identical to, those of Bcl-2. J Cell Biol 1995;128:1173–1184.
[3] Kozopas KM, Yang T, Buchan HL, Zhou P, Craig RW. MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. Proc Natl Acad Sci USA 1993;90:3516–3520.
[4] Krajewski S, Bodrug S, Krajewska M, Shabaik A, Gascoyne R, Berean K, et al. Immunohistochemical analysis of Mcl-1 protein in human tissues. Differential regulation of Mcl-1 and Bcl-2 protein production suggests a unique role for Mcl-1 in control of programmed cell death in vivo. Am J Pathol 1995;146:1309–1319.
[5] Akgul C, Moulding DA, White MR, Edwards SW. In vivo localisation and stability of human Mcl-1 using green fluorescent protein (GFP) fusion proteins. Fed Eur Biochem Soc Lett 2000;478:72–76.
[6] Fujise K, Zhang D, Liu J, Yeh ET. Regulation of apoptosis and cell cycle progression by MCL1. Differential role of proliferating cell nuclear antigen. J Biol Chem 2000;275:39458–39465.
[7] Akgul C, Turner PC, White MR, Edwards SW. Functional analysis of the human MCL-1 gene. Cell Mol Life Sci 2000;57:684–691.
[8] Moulding DA, Quayle JA, Hart CA, Edwards SW. Mcl-1 expression in human neutrophils: regulation by cytokines and correlation with cell survival. Blood 1998;92:2495–2502.
[9] Schubert KM, Duronio V. Distinct roles for extracellular-signal-regulated protein kinase (ERK) mitogen-activated protein kinases and phosphatidylinositol 3-kinase in the regulation of Mcl-1 synthesis. Biochem J 2001;356:473–480.
[10] Leuenroth SJ, Grutkoski PS, Ayala A, Simms HH. The loss of Mcl-1 expression in human polymorphonuclear leukocytes promotes apoptosis. J Leukoc Biol 2000;68:158–166.
[11] Wang X, Studzinski GP. Antiapoptotic action of 1,25-dihydroxyvitamin D3 is associated with increased mitochondrial MCL-1 and RAF-1 proteins and reduced release of cytochrome *c*. Exp Cell Res 1997;235:210–217.
[12] Pedersen IM, Kitada S, Leoni LM, Zapata JM, Karras JG, Tsukada N, et al. Protection of CLL B cells by a follicular dendritic cell line is dependent on induction of Mcl-1. Blood 2002;100:1795–1801.
[13] Moulding DA, Giles RV, Spiller DG, White MR, Tidd DM, Edwards SW. Apoptosis is rapidly triggered by antisense depletion of MCL-1 in differentiating U937 cells. Blood 2000;96:1756–1763.
[14] Derenne S, Monia B, Dean NM, Taylor JK, Rapp MJ, Harousseau JL, et al. Antisense strategy shows that Mcl-1 rather than Bcl-2 or Bcl-x(L) is an essential survival protein of human myeloma cells. Blood 2002;100:194–199.
[15] Miyamoto Y, Hosotani R, Wada M, Lee JU, Koshiba T, Fujimoto K, et al. Immunohistochemical analysis of Bcl-2, Bax, Bcl-X, and Mcl-1 expression in pancreatic cancers. Oncology 1999;56:73–82.
[16] Chung TK, Cheung TH, Lo WK, Yim SF, Yu MY, Krajewski S, et al. Expression of apoptotic regulators and their significance in cervical cancer. Cancer Lett 2002;180:63–68.
[17] Cho-Vega JH, Rassidakis GZ, Admirand JH, Oyarzo M, Ramalingam P, Paraguya A, et al. MCL-1 expression in B-cell non-Hodgkin's lymphomas. Hum Pathol 2004;35:1095–1100.
[18] Khoury JD, Medeiros LJ, Rassidakis GZ, McDonnell TJ, Abruzzo LV, Lai R. Expression of Mcl-1 in mantle cell lymphoma is associated with high-grade morphology, a high proliferative state, and p53 overexpression. J Pathol 2003;199:90–97.
[19] Backus HH, van Riel JM, van Groeningen CJ, Vos W, Dukers DF, Bloemena E, et al. Rb, mcl-1 and p53 expression correlate with clinical outcome in patients with liver metastases from colorectal cancer. Ann Oncol 2001;12:779–785.
[20] Jansen B, Zangemeister-Wittke U. Antisense therapy for cancer—the time of truth. Lancet Oncol 2002;3:672–683.
[21] Monia BP, Holmlund J, Dorr FA. Antisense approaches for the treatment of cancer. Cancer Invest 2000;18:635–650.
[22] Wacheck V, Heere-Ress E, Halaschek-Wiener J, Lucas T, Meyer H, Eichler HG, et al. Bcl-2 antisense oligonucleotides chemosensitize human gastric cancer in a SCID mouse xenotransplantation model. J Mol Med 2001;79:587–593.
[23] Thallinger C, Wolschek MF, Wacheck V, Maierhofer H, Gunsberg P, Polterauer P, et al. Mcl-1 antisense therapy chemosensitizes human melanoma in a SCID mouse xenotransplantation model. J Invest Dermatol 2003;120:1081–1086.
[24] Heere-Ress E, Thallinger C, Lucas T, Schlagbauer-Wadl H, Wacheck V, Monia BP, et al. Bcl-X(L) is a chemoresistance factor in human melanoma cells that can be inhibited by antisense therapy. Int J Cancer 2002;99:29–34.
[25] Thallinger C, Wolschek MF, Maierhofer H, Skvara H, Pehamberger H, Monia BP, et al. Mcl-1 is a novel therapeutic target for human sarcoma: synergistic inhibition of human sarcoma xenotransplants by a combination of mcl-1 antisense oligonucleotides with low-dose cyclophosphamide. Clin Cancer Res 2004;10:4185–4191.
[26] Wacheck V, Selzer E, Gunsberg P, Lucas T, Meyer H, Thallinger C, et al. Bcl-x(L) antisense oligonucleotides radiosensitise colon cancer cells. Br J Cancer 2003;89:1352–1357.
[27] Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Methods 1991;139:271–279.
[28] Burroughs A, Hochhauser D, Meyer T. Systemic treatment and liver transplantation for hepatocellular carcinoma: two ends of the therapeutic spectrum. Lancet Oncol 2004;5:409–418.
[29] Kobayashi S, Werneburg NW, Bronk SF, Kaufmann SH, Gores GJ. Interleukin-6 contributes to Mcl-1 upregulationand TRAIL resitance via an akt signaling pathway in cholangiocarcinoma cells. Gastroenterology 2005;128:2054–2065.
[30] Opferman JT, Iwasaki H, Ong CC, Suh H, Mizuno S, Akashi K, et al. Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. Science 2005;307:1101–1104.
[31] Reed JC. Apoptosis-based therapies. Nat Rev Drug Discov 2002;1:111–121.
[32] Geary RS, Leeds JM, Henry SP, Monteith DK, Levin AA. Antisense oligonucleotide inhibitors for the treatment of cancer: 1. Pharmacokinetic properties of phosphorothioate oligodeoxynucleotides. Anticancer Drug Des 1997;12:383–393.