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Mcl-1 Blocks Radiation-induced Apoptosis and Inhibits Clonogenic Cell Death

HANS SKVARA[1*], CHRISTIANE THALLINGER[2,3*], VOLKER WACHECK[2], BRETT P. MONIA[4], HUBERT PEHAMBERGER[3,6], BURKHARD JANSEN[2,5] and EDGAR SELZER[1,6]

[1]*Department of Radiotherapy and Radiobiology,*
[2]*Department of Clinical Pharmacology, Section of Experimental Oncology/Molecular Pharmacology and*
[3]*Department of Dermatology, Division of General Dermatology, University of Vienna,*
Waehringer Guertel 18-20, 1090 Vienna, Austria;
[4]*ISIS Pharmaceuticals Inc., 2292 Faraday Avenue, Carlsbad, CA 92008, U.S.A.;*
[5]*The Prostate Centre, University of British Columbia, 2733 Heather Street, Vancouver, BC V5Z 3J56, Canada;*
[6]*Center of Excellence for Clinical and Experimental Oncology (CLEXO), Austria*

Abstract. *Background: Anti-apoptotic Bcl-2 family proteins, such as Bcl-2 and Bcl-x, can modulate radio- and/or chemosensitivity of human malignancies. Since no information is available on the role Mcl-1 may play in the radioresponse of tumor cells, the relationship between Mcl-1 expression and response to ionizing radiation (IR) was investigated using an antisense strategy. Materials and Methods: Human melanoma cells were treated with Mcl-1 antisense oligonucleotides (ASOs) and IR. The effects of antisense treatment alone or in combination with IR on proliferation, induction of apoptosis and clonogenic cell death were evaluated. Results: ASO treatment in combination with IR reduced the mean cell numbers 9.5-fold compared to a 2.6-fold reduction after ASO treatment alone and a 1.6- fold reduction after IR alone. The percentages of apoptosis measured (means±SD) were 49%±3.0 in antisense/IR-treated cultures compared to 1.3%±0.5, 14.3%±0.5, 7.3%±1.1 and 10.3%±0.6 in ASO controls, in antisense-treated, in IR-treated and in antisense control plus IR-treated cells, respectively. Colony formation assays demonstrated a synergistic effect of Mcl-1 down-regulation with IR. Conclusion: Mcl-1 expression affects the radioresistance of human melanoma cells.*

*Hans Skvara and Christiane Thallinger should be regarded as equal contributors.

Correspondence to: Edgar Selzer, M.D., Department of Clinical Pharmacology, University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria. Tel: +43-1-40400-2981, Fax: +43-1-40400-2998, e-mail: edgar.selzer@meduniwien.ac.at

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Cellular propensity to radiation-induced death is critically affected by many well-investigated resistance factors. One of a variety of potential strategies to enhance treatment-induced tumor cell death is to modulate the expression of proteins, such as the anti-apoptotic Bcl-2 family members Bcl-2, Bcl-x, A1, Bcl-w or Mcl-1 (1-6), which in turn affect the apoptotic threshold of tumor cells. In addition to their role in regulating apoptosis, which can be induced by different treatments and conditions, these proteins are also thought to be involved in the control of other physiological processes, such as distribution of cells within the cell cycle or differentiation. The distribution of cells in the cell cycle, in turn, is able to affect radiosensitivity (4). Of the Bcl-2 family members mentioned above, the anti-apoptotic protein Mcl-1 seems to be an attractive candidate for further research, since it is expressed in a wide variety of tissues and cell types (7-9) Mcl-1 has some distinct biochemical characteristics compared to the other members of the anti-apoptotic Bcl-2 protein family, and its role in controlling cell survival has been shown in solid as well as in non-solid tumors. Mcl-1 is rapidly induced after exposure to various growth factors and has a very short half-life at the RNA and protein levels in the range of 2 to 3 h (10). In contrast to its more thoroughly investigated close relatives Bcl-2 and Bcl-xL, the role of Mcl-1 in the modulation of cell death, and specifically in the regulation of ionizing radiation-induced cell death, has not been investigated in such extensive detail. Significant interest in novel strategies influencing apoptotic cell death of human melanoma and other tumors has been raised in recent years. Antisense oligonucleotides (ASO) are chemically-modified stretches of single-stranded DNA. These are potentially powerful pharmacological inhibitors of protein expression (11, 12). They are specifically designed to bind to their

complementary mRNA sequence, resulting in inhibition of the expression of the protein of interest. Antisense strategies using ASO against Bcl-2, Bcl-xL and Mcl-1 have shown promise as potential treatment strategies for a variety of human malignancies (13-22). For instance, our group has previously reported the chemosensitizing effects of Bcl-2 ASOs in human melanoma, both pre-clinically and in clinical trials (14, 16, 22). Mcl-1 seems to be crucial for the survival of cells of hematological origin, such as myeloma cells, neutrophils and others (23-28). Human neutrophils, for example, express neither Bcl-2 nor Bcl-xL, but they do express Mcl-1. Mcl-1 down-regulation also chemosensitizes transplanted human sarcoma cells in a SCID mouse system (29). Upon shutdown of Mcl-1 expression, this pro-survival gene product is rapidly depleted, resulting in acceleration of apoptosis of human neutrophils. It has been reported that, in human melanoma, up-regulation of Mcl-1 is associated with malignant transformation (30). In conclusion, Mcl-1 is a key player in ensuring survival in different tumor entities by prolonging cell viability under various cytotoxic conditions. Mcl-1 down-regulation by antisense oligonucleotides, in order to facilitate treatment-induced cell death or to induce apoptotic cell death *per se,* has been used by several groups in different systems (see references above). Nevertheless, until now we are not aware of any reported experimental evidence connecting radiosensitivity with Mcl-1 expression. In the present study, the effects of decreasing Mcl-1 protein levels in human melanoma cells on their susceptibility towards treatment with ionizing radiation, were investigated for the first time.

Materials and Methods

Cell culture. The melanoma cell line 518A2 used for this study has been described previously (9). The cell line was maintained in Dulbecco's modified Eagle's culture medium(DMEM) supplemented with 10% fetal bovine serum (Gibco, Paisley, UK) and an antibiotic mixture (100 units/ml penicillin and 100 μg/ml streptomycin) in a fully humidified (5% CO_2, 95% ambient air) atmosphere at 37°C.

Oligonucleotides and transfection. 2'-O-Methoxyethyl/2'-deoxynucleotide chimeric phosphorothioate antisense oligonucleotides were kindly provided by ISIS Pharmaceuticals (Carlsbad, CA, USA). The sequence of Mcl-1 antisense oligonucleotide (ISIS 20408) was 5'-TTGGCTTTGTGTCC TTGGCG-3'. A universal control (UC) oligonucleotide pool (ISIS 29848) was used, which was synthesized from a mixture of A (adenine), G (guanine), T (thymine), and C (cytosine) bases resulting in a mixture of all possible oligonucleotide sequences. The oligonucleotide backbone chemistry of ISIS 29848 was identical to that of ISIS 20408. For transfection, 400,000 cells were seeded in a 75-cm^2 plate 24 h prior to oligonucleotide treatment. Oligonucleotides were complexed with lipofectin (Gibco) in an antibiotic-free medium without serum, as described by the supplier. Subsequently, cells were incubated for 4 h with 150-250 nM complexed oligonucleotides (10 μg per ml lipofectin) in antibiotic-free medium containing serum. After washing with DMEM, cells were further cultured in complete medium until termination of the experiment.

Determination of survival and apoptosis. For clonogenic survival experiments, subconfluent cells were treated with oligonucleotides at the concentrations indicated and then irradiated. Cells were treated by irradiation 24 h after transfection. Cells were then trypsinized and plated at appropriate predetermined dilutions onto 5-cm culture dishes. In general, between 200 and 2000 cells were seeded per dish in order to obtain between 100 and 300 single well-separated colonies per dish. After 8 to 12 days, cell culture plates were washed, fixed in 95% ethanol and stained with 0.1% crystal violet. Colonies containing more than 50 cells were scored as clonogenic survivors. The colony-forming experiments were performed three times for each treatment. Cells were irradiated with a conventional irradiation source. For immunofluorescence microscopy, 50,000 cells were grown on glass slides and treated with ASO (150 nM), as described above. Forty-eight h after irradiation, cells were trypsinized, washed with PBS and stained with Bisbenzimide (Hoechst 33342) from Sigma-Aldrich (Vienna, Austria). Apoptotic cells were then counted and photographed by immunofluorescence microscopy. For the assessment of cell growth, cells were incubated with oligonucleotides and/or exposed to ionizing radiation at the time-points and doses indicated. At various time-points after oligonucleotide treatment, the number of cells was determined using a Coulter Counter.

Western blot analysis. Cell extracts were prepared in lysis buffer containing 0.14 M NaCl, 0.4 M triethanolamine, 0.2% Na deoxycholate, and 0.5% Nonidet P-40, supplemented with 1 mM phenylmethylsulfonyl fluoride, 4.0 μg per ml aprotinin, and 4.0 μg per ml leupeptin. Total protein was quantified by means of a modified Bradford analysis (Bio-Rad, Richmond, CA, USA). Total lysates (20 μg per lane) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, blotted onto nitrocellulose membranes (Millipore, Bedford, MA, USA), and probed with anti-Mcl-1 (S-43), anti-Bax (P-19) and anti-Bak antibodies (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA). Bound antigen was visualized with the enhanced chemiluminescence detection system (ECL) from Boehringer Mannheim GmbH (Mannheim, Germany).

Statistical analysis. The statistical significance of differences was calculated using SigmaPlot 2002 for Windows Version 8.0 and the Student's *t*-test. *P* values below 0.05 were considered to be of statistical significance. Results are shown as means±standard deviations of 3 separate experiments.

Results

Mcl-1 expression in a variety of different human melanoma cell lines has recently been examined. By Western blot analysis; all human melanoma cell lines evaluated consistently expressed detectable levels of the anti-apoptotic protein Mcl-1 (9). Using Mcl-1 ASOs in the presence of the uptake enhancer lipofectin, a clear reduction in Mcl-1 levels was observed in melanoma cells *in vitro* at a concentration of 150 nM (Figure 1). Twenty-four h after addition of

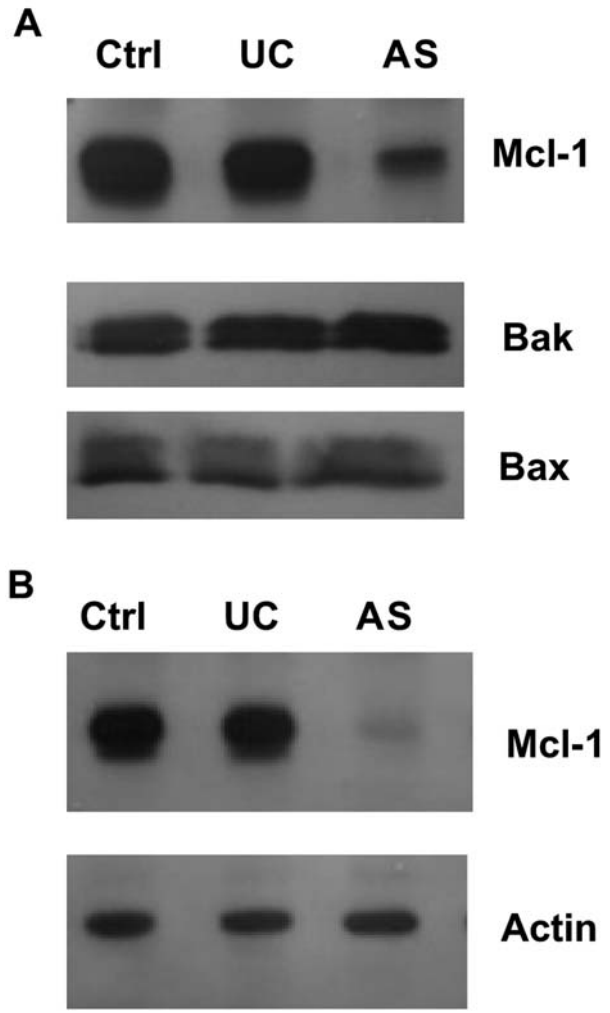

Figure 1. *Western blot analysis of Mcl-1 expression in ASO-treated melanoma cells. (A) Melanoma (518A2) 24 h after treatment with 150 nM MCL-1 ASOs in the presence of lipofectin; Ctrl, saline-treated control cells, UC, universal control oligonucleotides, AS, antisense oligonucleotide-treated cells. A representative blot of four independently performed experiments is presented. Bak and Bax control blots from the same lysates are shown in the panels below. (B) Mcl-1 ASO blot with increased concentrations (250 nM) of oligonucleotides and an actin blot from the same lysates serving as a control.*

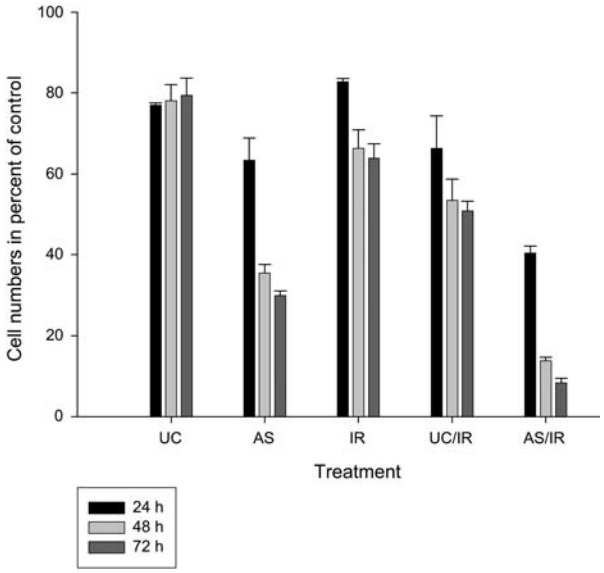

Figure 2. *Cell proliferation after different treatment combinations. Human melanoma cells were either untreated (Ctrl), or exposed to antisense (AS) or UC oligonucleotides. Cells were then irradiated and treated with ASOs as indicated. Irradiation (IR) was performed with a dose of 4 Gy. Cell growth was then determined by counting cell numbers 24h, 48h and 72 h after treatment. Data are means±SD of three independent experiments. Cell numbers are given in percent of untreated controls.*

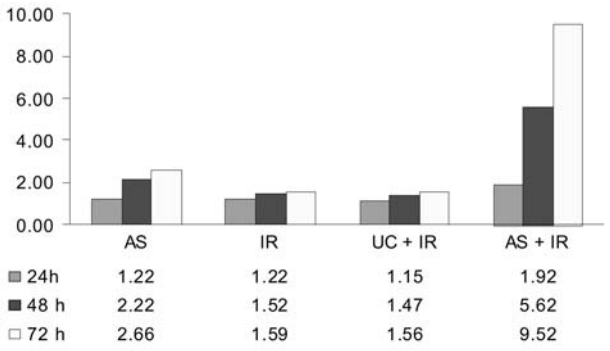

Figure 3. *Quantitative analysis of cell reduction. In short, mean values (in percent) of survival of untreated cells were divided by the mean values of survival of treated cells. Figure and table below demonstrate the extent of reduction in cell numbers (fold-reduction) by different treatment combinations obtained at various time-points.*

oligonucleotides to the cell culture, Mcl-1 levels in melanoma cells relative to lipofectin-treated control cells were reduced to 10-20% compared to the baseline expression levels (data not shown). Notably, treatment of melanoma cells with Mcl-1 ASO did not change Bak or Bax protein levels in the cell lines evaluated (Figure 1). At UC concentrations above 150 nM, a steep increase in unspecific cell death was observed. We have recently shown that the ASO-containing Mcl-1 with an identical sequence did not affect Bcl-2 or Bcl-x levels in human sarcoma cells (29). Time-course experiments using the same oligonucleotides showed a pronounced reduction of Mcl-1 protein, beginning from 6 to 8 h up to 36 hours after oligonucleotide treatment. Forty-eight h after oligonucleotide delivery, Mcl-1 expression returned to levels observed in untreated cells (29).

Next, the biological relevance of Mcl-1 expression as an anti-apoptotic protein and its potential role for radiosensitivity in human melanoma was addressed. For

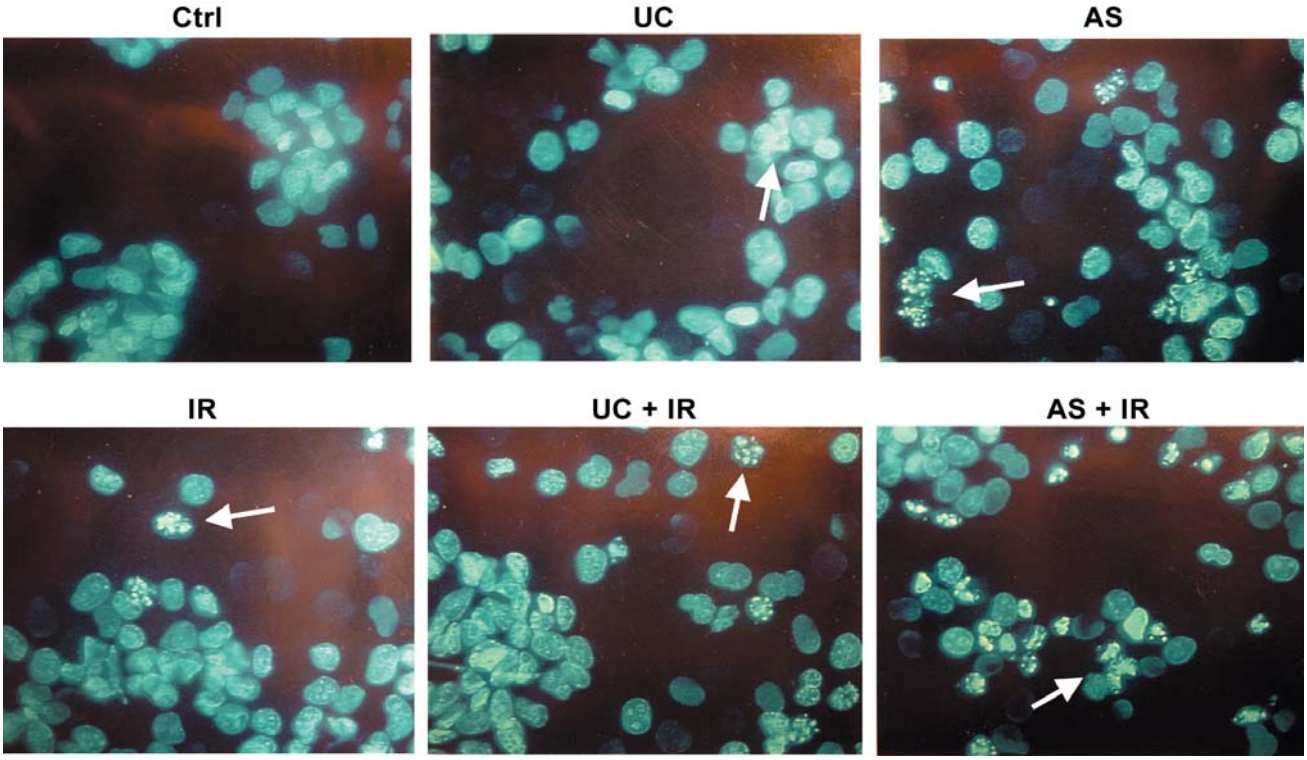

Figure 4. *Microscopic analysis of apoptosis 48 h after different treatments. Hoechst stain of melanoma cells. Cells were untreated (Ctrl) or treated with UC oligonucloetides, Mcl-1 ASOs alone or in combination with ionizing irradiation.*

these experiments, cells were transfected with Mcl-1 ASOs, universal control oligonucleotides or lipofectin alone. Following transfection, cells were cultured in complete medium and subsequently treated with ionizing radiation as indicated in the materials and methods section. Twenty-four h, 48 h and 72 h later, cell growth effects were evaluated by counting cell numbers. As shown in Figure 2, treatment with Mcl-1 ASOs alone, as well as ASO treatment plus IR, clearly decreased cell numbers compared to untreated or UC-treated cells. UC treatment alone reduced cell numbers by approximately 20% of control-treated cells, both in unirradiated or in irradiated cell cultures. Hence, there was no significant difference between irradiated and non-irradiated cell cultures after UC treatment. After 72 h, Mcl-1 ASO treatment alone, IR, and Mcl-1 ASO plus IR led to a reduction in cell numbers of 38%, 80% and 11%, respectively compared to UC-treated cells. After 48 h, the numbers were reduced to 46% (Mcl-1 ASO), 85% (IR) and 18% (Mcl-1 ASO plus IR), respectively, compared to untreated cells. These observations suggested a clear effect of the Mcl-1 ASO treatment on radiosensitivity. For a graphical representation and numerical analysis of the combination effects on survival, see Figure 3. Fold reduction of cell numbers was calculated using UC-treated cell numbers after the time-points indicated. After 72 h, for example, Mcl-1 ASO plus IR reduced cell numbers on average by a factor of 9.5, while IR alone or Mcl-1 ASO treatment alone reduced cell numbers 1.5 and 2.6-fold compared to UC-treated cells, suggesting a higher than additive effect of the combined treatment.

To assess whether the radiosensitization effects by Mcl-1 ASO were associated with an increased rate of early apoptotic cell death, treated cells were examined for morphological signs of apoptotic cell death by Hoechst staining, and the extent of apoptotic cell death was evaluated quantitatively. A photomicrograph of Hoechst-dye-stained cells, which were either untreated or treated with UC oligonucleotides, Mcl-1 ASOs, IR alone or in combination with Mcl-1 ASOs for 48 h, is provided. The percentage of apoptotic cells in UC-, ASO alone, IR-, UC plus IR- and ASO plus IR-treated cultures was 1.3% (SD 0.5), 14.3% (SD 0.5), 7.3% (SD 1.1), 10.3% (SD 0.6) and 49% (SD 3.0), respectively. Mcl-1 ASO plus IR therefore significantly and synergistically increased the number of apoptotic cells compared to Mcl-1 ASO- or IR-treated melanoma cell lines alone. An analysis of apoptotic cell

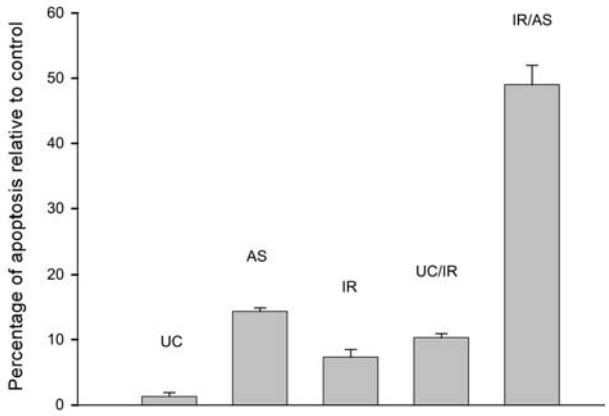

Figure 5. *Apoptosis after different treatments as indicated. UC, universal control oligonucleotide, AS, Mcl-1 antisense, IR, ionizing radiation. Fraction of apoptosis is given as the mean of three independent experiments ± SD.*

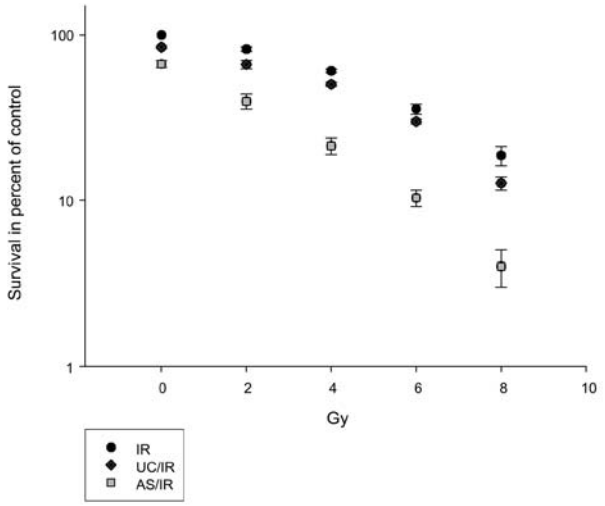

Figure 6. *Clonogenic survival analysis after Mcl-1 ASO treatment and/or irradiation. Melanoma cells were either left untreated, or were treated with a fixed concentration of ASOs. Twenty-four h later, cell culture dishes were treated with increasing doses of ionizing irradiation (0, 2, 4, 6 and 8 Gy) as indicated. Survival was assessed by performing colony-forming assays. Data are given as percentage survival of untreated cell cultures and represent the means (±SD) of three independent colony formation experiments.*

death after different treatments is shown in Figure 5. As can be seen from these data, the combination of Mcl-1 ASO with IR is clearly more effective in inducing apoptotic cell death compared to either treatment alone. Early apoptosis does not necessarily predict the clinically more relevant clonogenic survival sensitivity against genotoxic agents (31). Therefore, whether the early survival effects of Mcl-1 down-regulation would be reflected by a similarly reduced clonogenic survival was examined. As is shown in Figure 6, a combination of Mcl-1 ASO treatment with irradiation was more effective than either treatment alone. A dose-response analysis of survival in the presence of a fixed oligonucleotide concentration and increasing doses of irradiation demonstrated a decline of survival (measured in percent of untreated controls) after 0, 2, 4, 6 and 8 Gy irradiation alone to 82% (SD 2.6), 61% (SD 1.5), 36% (SD 2.5) and 19% (SD 2.5), respectively. In the presence of Mcl-1 ASOs, survival was further reduced by a factor (with increasing doses of irradiation) of 1.49 (67%, SD 3.2), 2.05 (40%, SD 4.1), 2.9 (21%, SD 2.5), 3.6 (10%, SD 1.1) and 4.75 (4%, SD 1.0), respectively. Differences in percentage of apoptosis between ASO-treated cells and control-treated cells were highly significant ($p \leq 0.0015$). Interestingly, our data show that, with increasing doses of IR, the radiosensitizing effects of down-regulation Mcl-1 expression were even more pronounced. On the other hand, the degree of unspecific effects of UC treatment on radiosensitivity were unaffected by irradiation dose, always being in the range of 20%. This observation provides additional support for the argument that the synergistic effect of Mcl-1 down-regulation on IR-induced clonogenic cell death by ASOs is indeed specific in nature.

Discussion

The influence of Mcl-1 expression on the sensitivity of human melanoma cells against radiotherapy was investigated by applying an antisense strategy. So far, only limited information has been available about the role Mcl-1 might play in the biology and treatment resistance of solid tumors, and virtually no information has been available about the role it might play in radioresistance. To the best of our knowledge, we showed for the first time that Mcl-1 expression is associated with the survival response against ionizing radiation. The importance of Mcl-1 expression was addressed, and the apoptotic threshold in human melanoma cells was lowered by down-regulating Mcl-1 protein by employing Mcl-1 ASOs. In our study, Mcl-1 ASOs previously shown to be capable of down-regulating Mcl-1 expression in endothelial and melanoma cells were used. *In vitro* experiments demonstrated a strong, rapid and specific down-regulation of the Mcl-1 target protein. The antisense effect was already observed after 8 h and lasted for 36 h. The observation that expression of Bax and Bak, as well as of Bcl-2 and Bcl-xL, which are closely related anti-apoptotic members of the Bcl-2 family, are not altered by Mcl-1 ASO treatment *in vitro* supports the notion that the effect of down-regulation of Mcl-1 on tumor growth is not due to accompanying changes in the expression of other Bcl-2 family members. Induction of apoptosis was accompanied in our experiments by a concomitant reduction of the cellular

survival of treated cells determined up to 72 h after the respective treatments. For example, an ~9-fold reduction of cell numbers was observed after combination treatment with Mcl-1 ASOs plus IR, in contrast to only a 2.6-fold reduction in survival after treatment with ASOs alone and a 1.6- fold reduction after IR alone. In agreement with these results, the percentage of apoptotic cells after combined treatment was 49%, while with ASO treatment alone 14% and with IR alone 7.3% apoptotic cells were counted. As has been pointed out by others, the induction of apoptosis must not necessarily translate into a direct measure of long-term treatment response against radiotherapy. For example, in prostate and other cancer cell types, Bcl-2 overexpression leads to a reduction in apoptosis after irradiation and/or chemotherapy without affecting clonogenic survival (31-34). On the other hand, our group has recently shown that antisense-mediated Bcl-xL down-regulation leads to reduced clonogenic cell death in colon cancer cells (35). We could show that a combination of RT and Mcl-1 down-regulation synergistically improved the results compared with each treatment alone. Taken together, we demonstrated that Mcl-1 down-regulation has the potential to enhance radiosensitivity in human melanoma *in vitro*. Treatment of melanoma *in vitro* with Mcl-1 ASOs followed by subsequent irradiation with clinically relevant doses resulted in increased tumor cell death when compared to both treatments alone. This approach carries not only the potential to increase the response rate of treatment-resistant human melanoma, but could also help to minimize the toxic side-effects of irradiation observed at standard doses, if locally applied radiotherapy is combined with systemic administration of ASOs. In conclusion, our data indicate, for the first time, a role for Mcl-1 as a radioresistance factor affecting early apoptotic response and proliferation as well as clonogenic survival. These findings suggest that a combination of Mcl-1 down-regulation with radiotherapy may qualify as a highly effective and promising treatment strategy in these, and most likely, in other types of cancer.

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References

1 Adams JM and Cory S: The Bcl-2 protein family: arbiters of cell survival. Science *281*: 1322-1326, 1998.

2 Oltvai ZN and Korsmeyer SJ: Checkpoints of dueling dimers foil death wishes. Cell *79*: 189-192, 1994.

3 Fujise K, Zhang D, Liu J and Yeh ET: Regulation of apoptosis and cell cycle progression by MCL1. Differential role of proliferating cell nuclear antigen. J Biol Chem *275*: 39458-39465, 2000.

4 Li L, Story M and Legerski RJ: Cellular responses to ionizing radiation damage. Int J Radiat Oncol Biol Phys *49*: 1157-1162, 2001.

5 Craig RW: MCL1 provides a window on the role of the BCL2 family in cell proliferation, differentiation and tumorigenesis. Leukemia *16*: 444-454, 2002.

6 Kozopas KM, Yang T, Buchan HL, Zhou P and Craig RW: MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to Bcl-2. Proc Natl Acad Sci USA *90*: 3516-3520, 1993.

7 Yang T, Kozopas KM and 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 *128*: 1173-1184, 1995.

8 Krajewski S, Bodrug S, Krajewska M *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 *146*: 1309-1319, 1995.

9 Selzer E, Schlagbauer-Wadl H, Okamoto I, Pehamberger H, Potter R and Jansen B: Expression of Bcl-2 family members in human melanocytes, in melanoma metastases and in melanoma cell lines. Melanoma Res *8*: 197-203, 1998.

10 Rogers S, Wells R and Rechsteiner M: Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science *234*: 364-368, 1986.

11 Jansen B and Zangemeister-Wittke U: Antisense therapy for cancer – the time of truth. Lancet Oncol *3*: 672-683, 2002.

12 Monia BP, Holmlund J and Dorr FA: Antisense approaches for the treatment of cancer. Cancer Invest *18*: 635-650, 2000.

13 Thallinger C, Wolschek MF, Wacheck V *et al*: Mcl-1 antisense therapy chemosensitizes human melanoma in a SCID mouse xenotransplantation model. J Invest Dermatol *120*: 1081-1086, 2003.

14 Zangemeister-Wittke U and Ziegler A: Bcl-2 antisense therapy for cancer: the art of persuading tumour cells to commit suicide. Apoptosis *3*: 67-74, 1998.

15 Gautschi O, Tschopp S, Olie RA *et al*: Activity of a novel bcl-2/bcl-xL-bispecific antisense oligonucleotide against tumors of diverse histologic origins. J Natl Cancer Inst *93*: 463-471, 2001.

16 Jansen B, Wacheck V, Heere-Ress E *et al*: Chemosensitisation of malignant melanoma by Bcl-2 antisense therapy. Lancet *356*: 1728-1733, 2000.

17 Webb A, Cunningham D, Cotter F *et al*: Bcl-2 antisense therapy in patients with non-Hodgkin lymphoma. Lancet *349*: 1137-1141, 1997.

18 Wacheck V, Heere-Ress E, Halaschek-Wiener J *et al*: Bcl-2 antisense oligonucleotides chemosensitize human gastric cancer in a SCID mouse xenotransplantation model. J Mol Med *79*: 587-593, 2001.

19 Heere-Ress E, Thallinger C, Lucas T *et al*: Bcl-X(L) is a chemoresistance factor in human melanoma cells that can be inhibited by antisense therapy. Int J Cancer *99*: 29-34, 2002.

20 Olie RA, Hafner C, Kuttel R *et al*: Bcl-2 and bcl-xL antisense oligonucleotides induce apoptosis in melanoma cells of different clinical stages. J Invest Dermatol *118*: 505-512, 2002.

21 Thallinger C, Wolschek MF, Wacheck V *et al*: Mcl-1 antisense therapy chemosensitizes human melanoma in a SCID mouse xenotransplantation model. J Invest Dermatol *120*: 1081-1086, 2003.

22 Jansen B, Schlagbauer-Wadl H, Brown BD *et al*: Bcl-2 antisense therapy chemosensitizes human melanoma in SCID mice. Nat Med *4*: 232-234, 1998.

23 Moulding DA, Quayle JA, Hart CA and Edwards SW: Mcl-1 expression in human neutrophils: regulation by cytokines and correlation with cell survival. Blood *92*: 2495-2502, 1998.
24 Zhou P, Qian L, Kozopas KM and Craig RW: Mcl-1, a Bcl-2 family member, delays the death of hematopoietic cells under a variety of apoptosis-inducing conditions. Blood *89*: 630-643, 1997.
25 Leuenroth SJ, Grutkoski PS, Ayala A and Simms HH: The loss of Mcl-1 expression in human polymorphonuclear leukocytes promotes apoptosis. J Leukoc Biol *68*: 158-166, 2000.
26 Moulding DA, Giles RV, Spiller DG, White MR, Tidd DM and Edwards SW: Apoptosis is rapidly triggered by antisense depletion of Mcl-1 in differentiating U937 cells. Blood *96*: 1756-1763, 2000.
27 Leuenroth SJ, Grutkoski PS, Ayala A and Simms HH: The loss of Mcl-1 expression in human polymorphonuclear leukocytes promotes apoptosis. J Leukoc Biol *68*: 158-166, 2000.
28 Zhang B, Gojo I and Fenton RG: Myeloid cell factor-1 is a critical survival factor for multiple myeloma. Blood *99*: 1885-1893, 2002.
29 Thallinger C, Markus F, Wolschek M *et al*: Mcl-1 is a novel target in human sarcoma: synergistic inhibition of human sarcoma by a combination of Mcl-1 antisense oligonucleotides with low-dose cyclophosphamide in SCID mice. Clin Cancer Res *10*: 4185-4191, 2004.
30 Tang L, Tron VA, Reed JC *et al*: Expression of apoptosis regulators in cutaneous malignant melanoma. Clin Cancer Res *4*: 1865-1871, 1998.
31 Kyprianou N, King ED, Bradbury D and Rhee JG: Bcl-2 over-expression delays radiation-induced apoptosis without affecting the clonogenic survival of human prostate cancer cells. Int J Cancer *70*: 341-348, 1997.
32 Breton C, Story MD and Meyn RE: Bcl-2 expression correlates with apoptosis induction but not loss of clonogenic survival in small cell lung cancer cell lines treated with etoposide. Anticancer Drugs *9*: 751-757, 1998.
33 Yin DX and Schimke RT: Bcl-2 expression delays drug-induced apoptosis but does not increase clonogenic survival after drug treatment in HeLa cells. Cancer Res *55*: 4922-4928, 1995.
34 Held KD: Radiation-induced apoptosis and its relationship to loss of clonogenic survival. Apoptosis *2*: 265-282, 1997.
35 Wacheck V, Selzer E, Gunsberg P *et al*: Bcl-x(L) antisense oligonucleotides radiosensitise colon cancer cells. Br J Cancer *89*: 1352-1357, 2003.

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