FEBS Letters 427 (1998) 124–128 FEBS 20176

APO2 ligand: a novel lethal weapon against malignant glioma?

Johannes Rieger[a], Ulrike Naumann[a], Tamara Glaser[a], Avi Ashkenazi[b], Michael Weller[a,*]

[a] *Laboratory of Molecular Neuro-Oncology, Department of Neurology, University of Tübingen, School of Medicine, Hoppe-Seyler-Strasse 3, 72076 Tübingen, Germany*

[b] *Department of Molecular Oncology, Genentech, Inc., South San Francisco, CA, USA*

Received 1 April 1998

Abstract APO2L (TRAIL) is a novel CD95L (Fas/APO-1-L) homologous cytotoxic cytokine that interacts with various receptors which transmit (DR4, DR5) or inhibit (DcR1, DcR2) an apoptotic signal. Here, we report that human glioma cell lines preferentially express mRNAs for agonistic death receptors DR4 (8/12) and DR5 (11/12) rather than the death-inhibitory decoy receptors DcR1 (4/12) and DcR2 (2/12). Ten of 12 cell lines are susceptible to APO2L-induced apoptosis. The resistant cell lines, U138MG and U373MG, are cross-resistant to CD95L-induced apoptosis. Similar to CD95L-induced apoptosis, APO2L-induced apoptosis is inhibited by ectopic expression of the caspase inhibitor, crm-A, or of bcl-2, or by coexposure to the corticosteroid, dexamethasone, or the lipoxygenase inhibitor, nordihydroguaretic acid. There is no correlation between p53 genetic status of the cell lines and their susceptibility to APO2L-induced apoptosis, but the latter is moderately enhanced by ectopic expression of wild-type p53. APO2L targeting may be a promising approach for selectively targeting apoptosis to human malignant glioma cells.

Key words: APO2 ligand; Apoptosis; Human glioma cell

1. Introduction

Targeting the tumor necrosis factor (TNF) receptor family member, CD95 (Fas/APO-1), by agonistic CD95 antibodies or the natural ligand, CD95 ligand (CD95L), is a promising therapeutic strategy for malignant glioma [1,2]. The APO2 ligand (APO2L), also referred to as TNF-related apoptosis-inducing ligand (TRAIL), is a novel member of the TNF cytokine family [3,4]. APO2L mRNA is expressed in most human tissues, including spleen, lung, and prostate, but not brain. APO2L is a potent inducer of apoptosis in different cancer cell lines. The first APO2L receptor to be cloned, DR4, showed sequence homology to the TNF receptor and to CD95 [5]. An additional APO2L receptor, DR5, also mediates APO2L-induced apoptosis [6,7]. Similar to CD95-mediated apoptosis, APO2L-induced apoptosis involves caspase activation [5–11]. Although DR4 is expressed in many human tissues including spleen and peripheral blood leukocytes, APO2L induces apoptosis in transformed cells but not in unstimulated primary T cells [10]. This can be explained by the co-expression of an antagonistic receptor, decoy receptor 1 (DcR1) or TRID (TRAIL receptor without intracellular domain), which is found in normal cells but is absent in most cancer cell lines [6,7]. Identification of another inhibitory receptor, DcR2 [12] or TRAIL-R4 [13], adds further complexity to the regulation of the APO2L/APO2L receptor system. In contrast to injection of CD95 antibodies and CD95L which is lethal to mice [14], injection of human APO2L is well-tolerated in mice (Ashkenazi, unpublished), suggesting that APO2L might be a relatively safe agent for cancer therapy. The present study examines the expression of APO2L receptors in human glioma cells and their susceptibility to APO2L-induced apoptosis.

2. Materials and methods

2.1. Materials and cell culture

APO2L was prepared as described [3]. Dexamethasone, NDGA and CHX were purchased from Sigma (Deisenhofen, Germany). T98G human malignant glioma cells were obtained from the American Type Culture Collection (Rockville, MD). A172, LN-229, LN-18, LN-308, LN-319, LN-428, U87MG, U138MG, U251MG, U373MG and D247MG human malignant glioma cells were kindly provided by Dr. N. de Tribolet (Lausanne, Switzerland). The glioma cells were cultured in DMEM containing 10% FCS, 2 mM glutamine, and 1% penicillin/streptomycin [1]. Soluble CD95L was derived from the supernatant of CD95L cDNA-transfected N2A murine neuroblastoma cells [15]. The amount of supernatant CD95L diluted in 100 μl medium that was necessary to kill 50% of LN-18 cells within 24 h was defined as one unit of CD95L.

Human glioma cell lines engineered to express murine bcl-2 or murine p53 Val^{135} and the respective neo or hygro control cell lines have been described [16,17]. A crm-A expression plasmid and puro control vector were obtained from Dr. A. Strasser [18]. All transfections were carried out using electroporation (Bio-Rad Gene Pulser, 250 V, 950 μF). Selection was done with G418 (neo, 0.5 mg/ml), hygromycin B (hygro, 200 μg/ml) or puromycin (puro, 2 μg/ml). Expression of the transgenes was ascertained by immunoblot analysis (bcl-2, p53) or Northern blot analysis (crm-A) (data not shown).

2.2. Survival and apoptosis assays

10 000 cells per well were seeded and treated as described, and survival was assessed by crystal violet staining in 96 well plates [1]. The experimental procedures for in situ DNA end labeling and quantification of DNA fragmentation have been published in detail elsewhere [19].

2.3. RT-PCR

Total RNA was isolated by guanidinium thiocyanate-phenol-chloroform extraction and treated with RNase-free DNase I (10 U/ml) for 30 min at 25°C to remove genomic DNA contamination. cDNA was synthesized from 5 mg RNA in a final volume of 20 μl using SuperScript II (Gibco-BRL, Gaithersburg, MD, USA) and oligo dT priming (Pharmacia, Uppsala, Sweden). The PCR conditions were as follows: DR4 35 cycles, 45 s/95°C, 45 s/60°C, 45 s/72°C, primer sequences CGATGTGGTCAGAGCTGGTACAGC (nucleotides 1182–1205) and GGACACGGCAGAGCCTGTGCCATC (nucleotides 1375–1398); DR5 35 cycles, 45 s/95°C, 45 s/60°C, 45 s/72°C, primer sequences GGGAGCCGCTCATGAGGAAGTTGG (nucleotides 1113–1136) and GGCAAGTCTCTCTCCCAGCGTCTC (nucleotides 1241–1264); DcR1 35 cycles, 45 s/95°C, 45 s/59°C, 45 s/72°C, primer sequences GTTTGTTTGAAAGACTTCACTGTG (nucleotides 963–986) and GCAGGCGTTTCTGTCTGTGGGAAC (nucleotides 1079–1102); DcR2 35 cycles, 45 s/95°C, 45 s/52°C, 45 s/72°C, primer sequences CTTCAGGAAACCAGAGCTTCCCTC (nucleo-

*Corresponding author. Fax: (49) (7071) 29 6507.
E-mail: michael.weller@uni-tuebingen.de

Abbreviations: CD95L, CD95 ligand; APO2L, APO2 ligand

PII S0014-5793(98)00409-8

tides 1330–1353) and TTCTCCCGTTTGCTTATCACACGC (nucleotides 1506–1529); β-actin 35 cycles, 45 s/95°C, 45 s/55°C, 45 s/72°C, primer sequences TGTTTGAGACCTTCAACACCC (nucleotides 409–429) and AGCACTGTGTTGGCGTACAG (nucleotides 918–937). The PCR fragments were separated in 2% agarose gels and visualized by ethidium bromide. The β-actin cDNA fragment was amplified as an internal control for equal amplification. A water control was run in each amplification to control for cross-contamination between tubes. No signal was obtained when RT was omitted during the RT step, confirming that the PCR signals were not derived from genomic DNA contamination.

2.4. Flow cytometry

CD95 expression at the cell surface was measured and quantified as SFI indices as outlined [20]. Briefly, 10^6 cells were trypsinized, centrifuged for 10 min at 4°C, stained with FITC-labeled anti-CD95 antibody (UB2, Immunotech, Hamburg, Germany) or FITC-labeled mouse IgG_1 (1 μg/ml) as a control, diluted in 100 μl PBS containing 1% bovine serum albumin and 0.01% sodium azide (flow cytometry buffer), and incubated for 30 min at 4°C. Thereafter, antibody was removed, cells washed twice in flow cytometry buffer, and resuspended in 300 μl PBS containing 1% paraformaldehyde and stored light-protected at 4°C until analysis in a FACScalibur (Becton-Dickinson, Heidelberg, Germany). CD95 expression is expressed as SFI (specific fluorescence index; mean fluorescence of specific antibody/mean fluorescence of isotype antibody).

2.5. Statistical analysis

Correlation analysis of APO2L receptor and CD95 expression was calculated by ANOVA. Correlation analysis of susceptibility to CD95L and APO2L was performed using Pearson correlation of survival fractions. Synergy between APO2L- and CD95L-induced apoptosis was assessed using the fractional product method of Webb as outlined in previous studies [15].

3. Results

3.1. Expression of APO2L receptors in human malignant glioma cell lines

The expression of DR4, DR5, DcR1 and DcR2 mRNAs was assessed by RT-PCR analysis (Fig. 1A). DR4 was expressed in eight of 12 cell lines. DR5 was expressed in all cell lines except for LN-428. In contrast, the decoy receptors DcR1 and DcR2 were only expressed in the minority of cell lines: DcR1 expression was detected in four cell lines (T98G, LN-319, LN-229, U251MG) whereas DcR2 expression was detected only in T98G and A172 cells. For comparison, CD95 protein expression in these cell lines was determined by flow cytometry (Fig. 1B). No correlation between the expression of CD95 and any APO2L receptor became apparent. For this analysis, the PCR data for APO2L receptor expression were rated as negative or positive, and related to CD95 expression as SFI data, or CD95 expression ranked high or low ($n = 6$ each).

3.2. Glioma cells are susceptible to APO2L-induced cell death

Next, the glioma cells were exposed to soluble APO2L (Fig. 2). For comparison, the cells were exposed to CD95L, and to combinations of APO2L and CD95L, in parallel. Further, these experiments were performed in the absence or presence of CHX because inhibition of protein synthesis has previously been shown to sensitize glioma cells to CD95L-induced apoptosis [1]. In the absence of CHX, APO2L killed LN-18, U87MG, LN-428, D247MG, T98G, LN-229, U251MG, LN-308 and A172 cells rather efficiently whereas U138MG and U373MG cells were resistant. Coexposure to APO2L and CHX enhanced APO2L-induced cytotoxicity especially at lower concentrations of APO2L, e.g. in U87MG, D247MG, LN-319, LN-229, U251MG, LN-308 and A172 cells. U138MG and U373MG cells were resistant even in the presence of CHX. APO2L-induced cell death showed typical features of apoptosis such as membrane blebbing and nuclear condensation as observed by phase contrast microscopy and

Table 1
APO2L-induced apoptosis of human malignant glioma cells: modulation by bcl-2, crm-A and p53

CHX	CD95L		APO2L	
	−	+	−	+
LN-18 neo	70 ± 2	42 ± 3	65 ± 2	30 ± 1
LN-18 bcl-2	92 ± 5*	55 ± 5*	93 ± 3*	40 ± 1*
LN-229 neo	98 ± 3	27 ± 3	75 ± 2	21 ± 4
LN-229 bcl-2	97 ± 7	67 ± 6*	83 ± 6	46 ± 5*
LN-18 puro	65 ± 0	29 ± 2	54 ± 4	34 ± 5
LN-18 crm-A	113 ± 1*	94 ± 7*	124 ± 3*	94 ± 4*
LN-229 puro	82 ± 2	14 ± 2	51 ± 1	9 ± 2
LN-229 crm-A	110 ± 2*	93 ± 3*	108 ± 3*	95 ± 4*
LN-18 hygro, 38.5°C	49 ± 2	37 ± 3	67 ± 2	46 ± 4
LN-18 p53, 38.5°C	42 ± 1	29 ± 1*	69 ± 3	31 ± 5*
LN-18 hygro, 32.5°C	102 ± 5	54 ± 4	76 ± 4	45 ± 3
LN-18, p53, 32.5°C	91 ± 5	36 ± 3*	50 ± 2*	33 ± 5*
LN-229 hygro, 38.5°C	89 ± 7	24 ± 2	69 ± 2	22 ± 1
LN-229 p53, 38.5°C	93 ± 4	13 ± 0*	70 ± 5	15 ± 1*
LN-229 hygro, 32.5°C	105 ± 10	31 ± 6	64 ± 8	26 ± 1
LN-229 p53, 32.5°C	91 ± 5	17 ± 2*	40 ± 7*	17 ± 2*

The cells were treated with CD95L (10 U/ml for LN-18, 100 U/ml for LN-229) or APO2L (0.1 μg/ml for LN-18, 1 μg/ml for LN-229). Survival assessed by crystal violet staining at 16 h. For the experiments assessing the role of p53 in wild-type conformation (32.5°C), the cells were preincubated for 4 h at 32.5°C before treatment. Data are expressed as mean percentages of survival and S.D. ($n = 3$, $^*P < 0.05$, t-test, significant difference compared with vector controls).

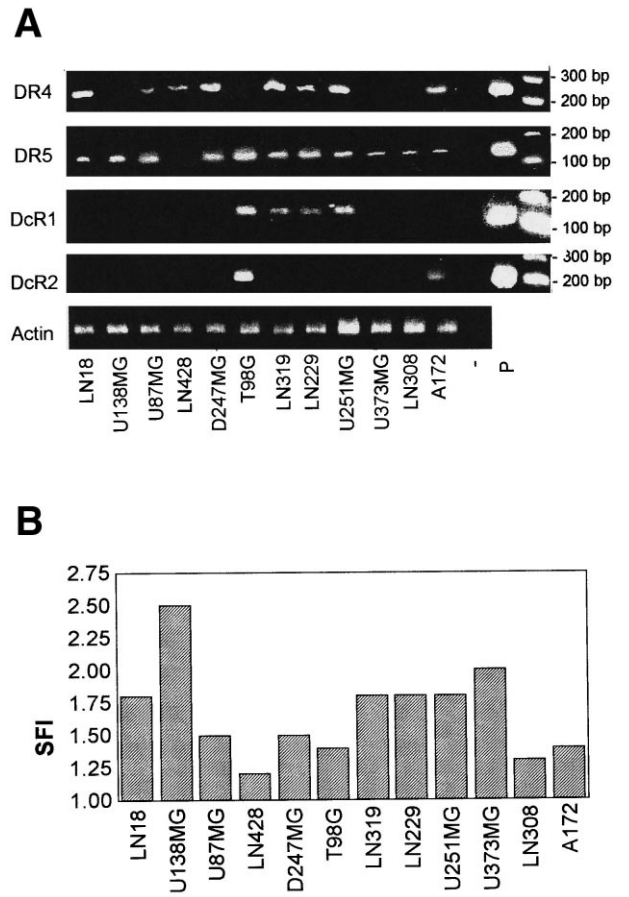

Fig. 1. Expression of APO2L receptors in human malignant glioma cell lines. A: DR4, DR5, DcR1 and DcR2 expression were analyzed by RT-PCR as outlined in Section 2. RNA quality was ascertained by parallel actin amplification (−, water control; P, cDNA plasmid control). The amplified fragment sizes were 217 bp for DR4, 152 bp for DR5, 140 bp for DcR1 and 200 bp for DcR1. B: CD95 expression was determined by flow cytometry. The SFI correponds to the ratio of fluoresence obtained with CD95 antibody versus isotype control antibody [20].

DNA breaks on in situ DNA end labeling (data not shown) as well as quantitative DNA fragmentation (Fig. 3A,B).

No clear relation between APO2L receptor expression at mRNA level and susceptibility to apoptosis became apparent. Both resistant cell lines, U373MG and U138MG, expressed DR5 but not DR4. However, both cell lines did not express the decoy receptors. On the other hand, expression of either DR4 or DR5 was sufficient to mediate apoptosis in LN-428, T98G and LN-308 cells.

A comparison of sensitivity to APO2L and CD95L showed some interesting parallels. Thus, LN-18 was most sensitive to both cytokines, and CHX did little to enhance cytotoxicity in these cells. The APO2L-resistant cell lines U138MG and U373MG were also resistant to CD95L. Survival fractions after exposure to CD95L (100 U/ml) correlated significantly with the survival fractions after exposure to APO2L (1 μg/ml) ($r = 0.73$, $P < 0.05$). According to the fractional product method, the coexposure to APO2L and CD95L (Fig. 2) had no synergistic activity except in A172 cells with the lower cytokine concentrations and in the absence of CHX (79.6% predicted survival, 59.2% factual survival). To confirm that APO2L does not interact with CD95 expressed on the glioma cells, we showed that LN-308 and LN-319 cells sensitized to CD95L-induced apoptosis by CD95 gene transfer [20] were

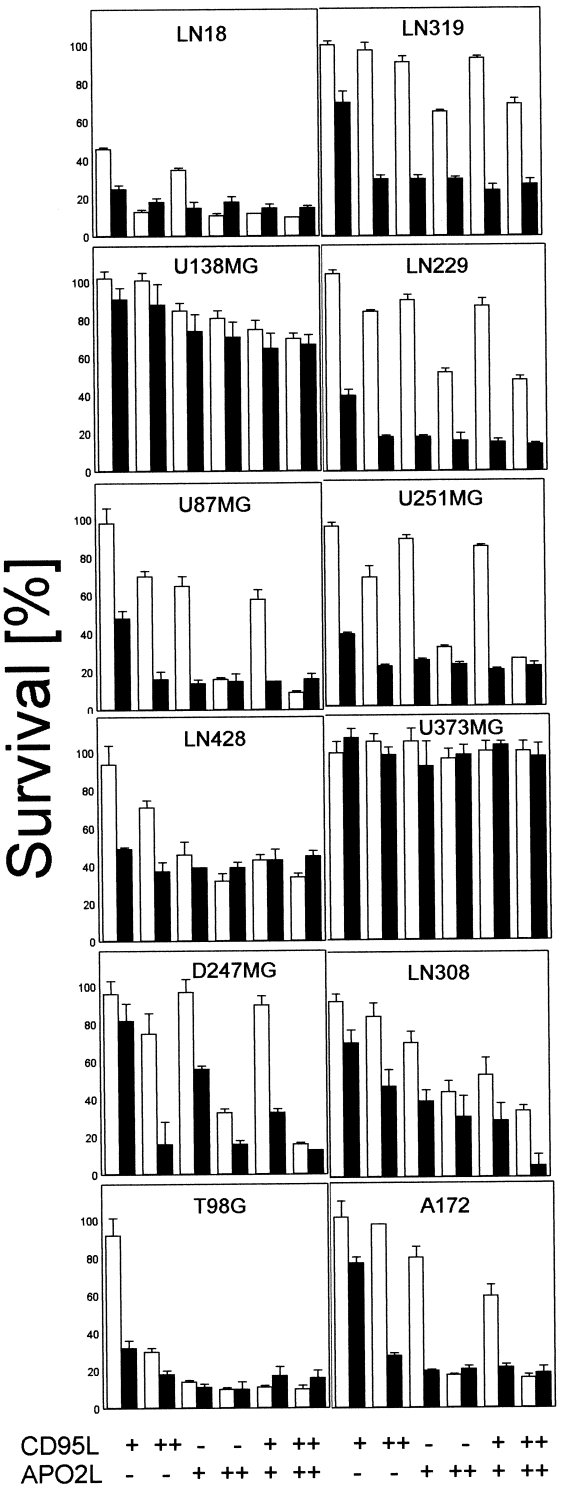

Fig. 2. APO2L-induced apoptosis of human malignant glioma cells: comparison with CD95L. The cells were exposed to CD95L at 10 (+) or 100 (++) U/ml, to APO2L at 0.1 (+) or 1 (++) μg/ml, or combinations of CD95L (10 U/ml) plus APO2L (0.1 μg/ml) or of CD95L (100 U/ml) plus APO2L (1 μg/ml), in the absence (open bars) or presence (filled bars) of cycloheximide (10 μg/ml) for 16 h. Survival was asssessed by crystal violet staining. Data are expressed as mean percentages of survival and S.D. ($n = 3$).

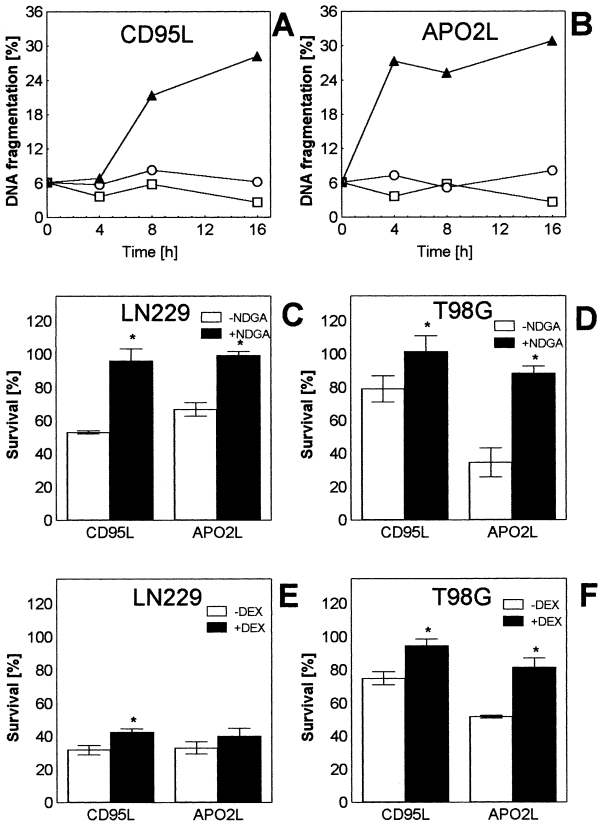

Fig. 3. Death ligand-induced DNA fragmentation in human malignant glioma cells and cytoprotective effects of dexamethasone and NDGA. A, B: LN-229 cells were treated with CD95L (100 U/ml, A, left) or APO2L (1 μg/ml, B, right) in the absence or presence of CHX (10 μg/ml) for 4, 8 or 16 h (open circles, death ligand alone; open squares, CHX alone; filled triangles, death ligand plus CHX). Data are expressed as mean percentages of DNA fragmentation ($n=3$, S.E.M. $<10\%$). C–F: LN-229 (C, E) or T98G cells (D, F) were exposed to CD95L (100 U/ml) or APO2L (1 μg/ml) in the absence (open bars) or presence (filled bars) of NDGA (20 μM) (C, D) or dexamethasone (100 nM) (DEX, E, F). Data are expressed as mean percentages and S.E.M. of survival assessed by crystal violet staining ($n=3$, $^*P<0.05$, t-test).

not sensitized to APO2L-induced apoptosis (data not shown).

3.3. Genetic modulation of APO2L-induced apoptosis in human glioma cells

Next, we characterized the subcellular pathway of APO2L-induced apoptosis in glioma cells (Table 1). Similar to CD95L-induced apoptosis [16,21], APO2L-induced cell death was attenuated by ectopic expression of bcl-2. Since caspase activation is an important step in CD95-mediated apoptosis in non-glial cells, we generated glioma cell sublines expressing the viral caspase inhibitor, crm-A. Both CD95L- and APO2L-induced apoptosis were completely blocked by crm-A. The essential requirement of caspase activity for apoptosis was confirmed by pharmacological experiments using the pseudosubstrate peptide inhibitor, DEVD-cho which completely blocked APO2L-induced cell death at 400 μM (data not shown).

We have previously reported that CD95L-induced glioma cell apoptosis is attenuated by dexamethasone and the lipoxygenase inhibitor, NDGA [1,22]. A similar degree of protection from apoptosis was observed when APO2L was used as a death stimulus even though dexamethasone-mediated protection failed to reach significance in LN-229 cells (Fig. 3C–F).

Of the 12 human glioma cell lines, four retain wild-type p53 activity (LN-229, U87MG, D247MG, U138MG) whereas eight are mutant or deleted for p53 [23]. Fig. 2 indicates that the p53 status has no impact on the sensitivity to APO2L or CD95L. Since DR5 expression is up-regulated by wild-type p53 in non-glial cells [24], we examined APO2L-induced apoptosis in LN-18 and LN-229 cells which were transfected with temperature-sensitive p53 Val^{135} which is mutant at 38.5°C and assumes wild-type conformation at 32.5°C [17]. Table 1 shows that the ectopic expression of mutant (38.5°C) p53 does not significantly alter CD95L- or APO2L-induced apoptosis in the absence of CHX. In the presence of CHX, p53-transfected cells are moderately more sensitive to apoptosis induced by both death ligands. Wild-type p53 enhances apoptosis induced by both ligands both in the absence and presence of CHX. However, these effects are small and of questionable biological significance.

4. Discussion

Human malignant gliomas are rather resistant to current therapeutic approaches which include cytoreductive surgery, irradiation, and adjuvant chemotherapy. However, these cells are not resistant to CD95L-induced apoptosis which is therefore a promising experimental strategy to eliminate human glioma cells in vivo [2]. The major problem with CD95 targeting in vivo is not lack of efficacy but serious concerns about potential toxicity [14]. Given the complex regulation of the APO2L/APO2L receptor system with regard to differential expression of agonistic and inhibitory receptors, we examined whether the APO2L system might be more suitable for the experimental therapy of gliomas in vivo than the CD95/CD95L system. Expression of DR4 in the normal brain has not been examined whereas DR5, DcR1 and DcR2 expression was not detected by Northern blotting in normal brain [6,7].

We find that most human glioma cell lines express either DR4 or DR5, or both, agonistic APO2L receptors whereas few cell lines express the antagonistic receptors DcR1 and DcR2 (Fig. 1). Further, 10 of 12 cell lines are rather susceptible to APO2L-induced apoptosis, notably in the presence of CHX (Fig. 2). There was considerable overlap between the sensitivity or resistance patterns of the glioma cell lines to APO2L and CD95L. The biochemical pathways of apoptosis activated by both death ligands were very similar: bcl-2 and caspase inhibitors were protective, as were dexamethasone and the lipoxygenase inhibitor NDGA (Table 1, Fig. 3). Further, sensitivity to both death ligands showed no dependence on p53 genetic status, and ectopic expression of wild-type p53 moderately enhanced the cytotoxic effects of both ligands, consistent with p53-mediated induction of DR5 expression in non-glial cells [24]. Thus, the death cascades triggered by CD95 and DR4/DR5 converge early and are likely to be identical downstream. A defect in the death domain-induced signalling cascade might be responsible for the resistance of two cell lines, U138MG and U373MG. Given the more complex regulation of APO2L receptors, with death-inhibitory receptors expressed preferentially by non-neoplastic cells, APO2L targeting may be an equally effective but safer approach of targeting therapeutic apoptosis to human malignant glioma cells.

Acknowledgements: This work was supported by Deutsche Forschungsgemeinschaft (We 1502/5-2).

References

[1] Weller, M., Frei, K., Groscurth, P., Krammer, P.H., Yonekawa, Y. and Fontana, A. (1994) J. Clin. Invest. 94, 954–964.
[2] Weller, M., Kleihues, P., Dichgans, J. and Ohgaki, H. (1998) Brain Pathol. (in press).
[3] Pitti, R.M., Marsters, S.A., Ruppert, S., Donahue, C.J., Moore, A. and Ashkenazi, A. (1996) J. Biol. Chem. 271, 12687–12690.
[4] Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C.-P., Nicholl, J.K., Sutherland, G.R., Smith, T.D., Rauch, C., Smith, C.A. and Goodwin, R.G. (1995) Immunity 3, 673–682.
[5] Pan, G., O'Rourke, K., Chinnaiyan, A.M., Gentz, R., Ebner, R., Ni, J. and Dixit, V.M. (1997) Science 276, 111–113.
[6] Sheridan, J.P., Marsters, S.A., Pitti, R.M., Gurney, A., Skubatch, M., Baldwin, D., Ramakrishnan, L., Gray, C.L., Baker, K., Wood, W.I., Goddard, A.D., Godowski, P. and Ashkenazi, A. (1997) Science 277, 818–821.
[7] Pan, G., Ni, N., Wei, Y.-F., Yu, G.-L., Gentz, R. and Dixit, V.M. (1997) Science 277, 815–818.
[8] Chaudhary, P.M., Eby, M., Jasmin, A., Bookwalter, A., Murray, J. and Hood, L. (1997) Immunity 7, 821–830.
[9] Schneider, P., Thome, M., Burns, K., Bodmer, J.-L., Hofmann, K., Kataoka, T., Holler, N. and Tschopp, J. (1997) Immunity 7, 831–836.
[10] Marsters, S.A., Pitti, R.M., Donahue, C.J., Ruppert, S., Bauer, K.D. and Ashkenazi, A. (1996) Curr. Biol. 6, 750–750.
[11] Mariani, S.M., Matiba, B., Armandola, E.A. and Krammer, P.H. (1997) J. Cell Biol. 137, 221–229.
[12] Marsters, S.A., Sheridan, J.P., Pitti, R.M., Huang, A., Skubatch, M., Baldwin, D., Yuan, J., Gurney, A., Goddard, A.D., Godowski, P. and Ashkenazi, A. (1997) Curr. Biol. 7, 1003–1006.
[13] Degli-Esposti, M.A., Dougall, W.C., Smolak, P.J., Waugh, J.Y., Smith, C.A. and Goodwin, R.G. (1997) Immunity 7, 813–820.
[14] Ogasawara, J., Watanabe-Fukunaga, R., Adachi, M., Matsuzawa, A., Kasugai, T., Kitamura, Y., Itoh, N., Suda, T. and Nagata, S. (1993) Nature 364, 806–809.
[15] Roth, W., Fontana, A., Trepel, M., Dichgans, J., Reed, J.C. and Weller, M. Cancer Immunol. Immunother. 44, 55–63.
[16] Weller, M., Malipiero, U., Aguzzi, A., Reed, J.C. and Fontana, A. (1995) J. Clin. Invest. 95, 2633–2643.
[17] Trepel, M., Groscurth, P., Malipiero, U., Gulbins, E., Dichgans, J. and Weller, M. (1998) J. Neuro-Oncol. (in press).
[18] Strasser, A., Harris, A.W., Huang, D.C., Krammer, P.H. and Cory, S. (1995) EMBO J. 14, 6136–6147.
[19] Weller, M., Trepel, M., Grimmel, C., Schabet, M., Bremen, D., Krajewski, S. and Reed, J.C. (1997) Neurol. Res. 19, 459–470.
[20] Weller, M., Malipiero, U., Rensing-Ehl, A., Barr, P.J. and Fontana, A. (1995) Cancer Res. 55, 2936–2944.
[21] Roth, W., Wagenknecht, B., Grimmel, C., Dichgans, J. and Weller, M. (1998) Br. J. Cancer 77, 404–411.
[22] Wagenknecht, B., Gulbins, E., Lang, F., Dichgans, J. and Weller, M. (1997) FEBS Lett. 409, 17–23.
[23] Van Meir, E.G., Kikuchi, T., Tada, T., Li, H., Diserens, A.C., Wojcik, B.E., Huang, H.J.S., Friedmann, T., De Tribolet, N. and Cavenee, W.K. (1994) Cancer Res. 54, 649–652.
[24] Wu, G.S., Burns, T.F., McDonald, E.R., Jiang, W., Meng, R., Krantz, I.D., Kao, G., Gan, D.D., Zhou, J.Y., Muschel, R., Hamilton, S.R., Spinner, N.B., Markowitz, S., Wu, G. and Deiry, W.S. (1997) Nature Genet. 17, 141–143.