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Dexamethasone inhibits TRAIL-induced apoptosis of thyroid cancer cells via Bcl-x_L induction

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ABSTRACT

We have investigated the effect of dexamethasone (DEX) on the apoptosis induced by TRAIL (tumour necrosis factor-related apoptosis inducing ligand) in follicular undifferentiated thyroid (FRO) cancer cells. Apoptosis was measured by percent hypodiploid nuclei, caspase-3 and -8 activation, and mitochondrial membrane depolarisation. DEX nearly abolished TRAIL-induced apoptosis. The DEX protective effect was reverted by the steroid receptor antagonist RU486 suggesting that the DEX action is mediated by glucocorticoid receptor (GR) activation. The role of Bcl proteins in the DEX effect was then investigated. In FRO cells DEX stimulated in a time-dependent fashion the expression of Bcl-x_L, but not that of Bcl-2, Bax and Bad. In addition, Bcl-x_L mRNA was significantly increased in the presence of DEX, suggesting a transcriptional regulation by the steroid. Transfection of the cells with siRNAs against Bcl-x_L inhibited both basal and DEX-stimulated Bcl-x_L expression and restored apoptosis in TRAIL-stimulated cells treated with DEX. These results demonstrate that dexamethasone protects thyroid cancer cells from apoptosis induced by TRAIL. DEX acts via GR activation and up-regulation of the expression of the anti-apoptotic protein Bcl-x_L.

1. Introduction

Apoptosis is a physiological cell death process in which cells are removed from the body without eliciting an inflammatory response. During apoptosis a number of specific morphological alterations take place, including cell shrinkage, chromatin condensation, DNA fragmentation with the formation of apoptotic bodies. Apoptotic cells and bodies are rapidly recognised and engulfed by neighbouring cells and phagocytes, thereby preventing complications that would result from the release of intracellular content. Dysregulation of apoptosis can lead to severe pathological syndromes. Neurodegenerative disorders may result from accelerated neuronal apoptosis, while escape of transformed cells from the apoptotic death program may lead to neoplastic proliferation. Thus, manipulation of apoptosis by experimental compounds has been in recent years an area of large interest to develop novel therapeutic strategies.[1]

Selective induction of apoptosis in malignant cells may represent an attractive mechanism to control neoplastic cell proliferation. Tumour necrosis factor-related apoptosis inducing ligand (TRAIL) is a member of the tumour necrosis factor (TNF) family including FasL, and TNF-α. TRAIL induces apoptosis by interacting with two cell-surface receptors, death

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receptor (DR)4 (TRAIL-R1) and DR5 (TRAIL-R2).[2] Since TRAIL is able to induce apoptosis in many transformed and malignant cells, but not in normal cells[3] it has raised strong interest as a new promising anti-cancer agent. TRAIL receptors are broadly expressed in thyroid carcinomas and thyroid carcinoma cell lines.[4] TRAIL effectively kills most thyroid cancer cell lines tested, including those originating from anaplastic carcinomas.[4,5] The anticancer activity of TRAIL appears to be independent of p53, suggesting that it can be effective against poorly differentiated cancer cells.[4] We have recently shown that apoptosis induced by TRAIL in follicular undifferentiated thyroid cancer cells is partially mediated by up-regulation of the expression of annexin-1 which, in turn, may act by increasing Bad dephosphorylation. This allows Bad to associate with mitochondria, heterodimerise with Bcl-x_L. and promote apoptosis.[6]

Glucocorticoid (GC) hormones can either promote or inhibit apoptosis according to cell type.[7] In tumours of lymphoid tissues and organs glucocorticoids are frequently used as cotreatment because they may have potent proapoptotic properties and reduce nausea, hyperemesis, and acute toxicity on normal tissues. In contrast, in a number of solid tumours, glucocorticoids may inhibit apoptosis by different stimuli raising questions on the use of these compounds as adjuvant therapy in cancer.[8]

On the basis of the above considerations we decided to investigate the effect of dexamethasone (DEX), a potent glucocorticoid agent, on the apoptosis induced by TRAIL in follicular undifferentiated thyroid (FRO) cancer cells. We report that DEX nearly abolished TRAIL-induced apoptosis via receptor activation and up-regulation of the anti-apoptotic protein Bcl-x_L expression. Bcl-x_L up-regulation appears to contribute to the DEX-mediated protection from apoptosis in thyroid cancer cells through a pathway that inhibits TRAIL-induced mitochondrial depolarisation.

2. Materials and methods

2.1. Drugs

Dexamethasone 21-phosphate disodium salt (DEX, Sigma-Aldrich, Gallarate, Milan, Italy) and soluble human recombinant SuperKiller TRAIL (Alexis Corporation, Vinci-Biochem, Florence, Italy) were dissolved in culture medium Dulbecco's modified Eagle's medium (DMEM) to obtain the desired concentrations. Mifepristone (RU486, Sigma-Aldrich) was dissolved in 100% ethanol to make a stock solution of 10^{-3} M, which was then diluted in culture medium to obtain the desired concentration.

2.2. Cell culture

Papillary carcinoma cells (NPA), follicular carcinoma cells (WRO), follicular undifferentiated thyroid cancer cells (FRO) were cultured in DMEM supplemented with 2 mM L-glutamine, 10% heat-inactivated foetal bovine serum (FBS), 10000 units/ml penicillin and 10 mg/ml streptomycin (all from Cambrex Bioscience, Verviers, Belgium) at 37 °C in an atmosphere of 95% O_2 and 5% CO_2, as previously described.[9] The most undifferentiated thyroid carcinoma cells (ARO) were cultured in RMPI supplemented with 2 mM L-glutamine, 10% heat-inactivated foetal bovine serum (FBS), 10,000 units/ml penicillin and 10 mg/ml streptomycin. In this study, the cells were plated at a density of 1×10^6 cells/well in 10-cm cell culture plates (Falcon, BD Bioscience, Bedford, USA) the day before treatment. At the end of the various incubation treatments, as described in the Results section, the cells were processed for Western blotting and FACS analyses. The cells were used up to maximum of 15 passages.

2.3. Analysis of apoptosis

Hypodiploid DNA was analysed using the method of propidium iodide (PI) staining and flow cytometry as described.[10] Briefly, cells were washed in phosphate-buffered saline (PBS) and resuspended in 500 μl of a solution containing 0.1% sodium citrate, 0.1% Triton X-100 and 50 μg/ml PI (Sigma-Aldrich). After incubation a 4 °C for 30 min in the dark, cell nuclei were analysed with Becton Dickinson FACScan flow cytometer using the Cells Quest program. Cellular debris was excluded from analysis by raising the forward scatter threshold, and the DNA content of the nuclei was registered on a logarithmic scale. The percentage of cells in the hypodiploid region was calculated.

2.4. Analysis of mitochondrial membrane potential ($\Delta\psi_m$)

Loss of $\Delta\psi_m$ was assessed using fluorescence-activated cell sorter (FACS) analysis of cells stained with tetramethylrhodamine ethyl ester (TMRE, Sigma-Aldrich), as previously described.[11] Cells were incubated with 50 nM TMRE for 15 min at 37 °C before starting drug treatment. For FACS analysis, cells were harvested by trypsinisation at the end of experimental protocols and analysed by Becton Dickinson FACScan flow cytometer (20,000 cells/sample). The fluorescence intensity of TMRE was monitored at 582 nm (FL-2).

2.5. Western blotting analysis

Total intracellular proteins were extracted from the cells by freeze/thawing in lysis buffer (50 mM Trizma-HCl). Protein content was estimated according to Biorad protein assay (BIO-RAD, Milan, Italy) and the samples either analysed immediately or stored at −80 °C. Samples (50 μg protein) were loaded onto 12% acrylamide gel and separated by SDS–PAGE in denaturating conditions at 50 V. The separated proteins were then transferred electrophoretically (100 mA per blot 90 min; Trans Blot Semi-Dry, BIO-RAD) to nitrocellulose paper (Immobilon-NC, Millipore, Bedford, USA) soaked in transfer buffer (25 mM Tris, 192 mM glycine, Sigma-Aldrich) and 20% methanol vol/vol (Carlo Erba, Milan, Italy). Non specific binding was blocked by incubation of the blots in 5% no fat dry-milk powder (BIO-RAD) in TBS/0.1%Tween (25 mM Tris; 150 mM NaCl; 0.1% Tween vol/vol, Sigma-Aldrich) for 60 min. After washing, the blots were incubated overnight at 4 °C with the primary mouse monoclonal anti-Bcl-x_L (diluted 1:750), mouse monoclonal anti-Bcl-2 (diluted 1:750), rabbit polyclonal anti-Bax (diluted 1:1000), rabbit polyclonal anti-caspase-3 (diluted 1:1000) (all from Santa-Cruz Biotechnology, D.B.A. ITALIA s.r.l, Milan, Italy), rabbit polyclonal anti-BAD and mouse

monoclonal anti-caspase-8 antibodies (Cell Signaling Technology, Inc. Danvers, USA) (diluted 1:1000). After incubation with the primary antibodies and washing in TBS/0.1% Tween, the appropriate secondary antibody, either anti-mouse (diluted 1:1000), or anti-rabbit (diluted 1:5000) (Sigma-Aldrich) was added for 1 h at room temperature. Immunoreactive protein bands were detected by chemiluminescence using enhanced chemiluminescence reagents (ECL) and exposed to Hyperfilm (both from Amersham Biosciences, Milan, Italy). Films were then subjected to densitometric analysis using a Gel-Doc 2000 system (BIO-RAD).

2.6. RNA extraction and Real-time quantitative PCR analysis

Total RNA was extracted from cell cultures after different times of incubation with DEX 10 μM (0, 4, 8 h), using TRIzol reagent (Invitrogen, Milan, Italy). 1 μg RNA from each sample was treated with DNase I (Invitrogen) and reverse transcribed using oligo (dT) primers and Superscript First Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer instructions. Real-time PCR amplifications and analyses were performed on a Light-cycler system (Roche) using the LightCycler–FastStart DNA Master plus SYBR Green I kit (Roche Molecular Biochemicals, Mannheim, Germany). Bcl-x_L and the house-keeping gene GAPDH were amplified with specific primers. Sense primer for Bcl-x_L was 5′-ATCAATGGCAACCCATCCTG-3′, corresponding to nucleotides 285–304 and antisense primer was 5′-TTGTCTACGCTTTCCACGCA-3′, corresponding to nucleotides 584–604 of human Bcl-x_L cDNA sequence. Sense primer for GAPDH was 5′-ACCCACTCCTCCACCTTTG-3′ corresponding to nucleotides 928–946 and antisense primer was 5′-CTCTTGTGCTCTTGCTGGG-3′ corresponding to nucleotides 1087–1105 of human GAPDH cDNA sequence. The PCR protocol for both genes was as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 10 s, annealing at 55 °C for 5 s and extension at 72 °C for 10 s.

Standard curves were generated from increasing amount of cDNA made from total control RNA. The C_T values were used to calculate a linear regression line by plotting the logarithm of template concentration (x-axis) against the C_T value (y-axis). These regression lines were used to calculate the expression level (nanogram of total RNA) for unknown samples. Expression levels of Bcl-x_L were normalised with GAPDH mRNA in each sample. All experiments were performed in triplicate.

2.7. siRNA oligonucleotides preparation and transfection

The siRNA against Bcl-x_L was siGENOME SMARTpooll reagent M-003458-00-0005, 5 nmol, BCL2L1 (Dharmacon Research Inc, Lafayette, CO). siRNA Oligo Scrambled KROAA-006461 (sense 5′-CAGUCGCGUUUGCGACUGG-3′, Dharmacon) was used as control. According to the manufacturer's instructions these RNAs were diluted with sterile Rnase-free water at 50 nM final concentration. Approximately, 1×10^5 FRO cells were plated in six-well plates at 30–50% confluency in media containing 10% FBS. Transfection of siRNAs was performed using oligofectamine (Invitrogen) as previously described.[6] 48 h after transfection cells were treated with DEX (10 μM) for 4 h before adding TRAIL (5 ng/ml) for different times. At the end of the incubation periods the cells were processed for Western blotting and FACS analyses.

2.8. Statistical analysis

All results are shown as mean ± SEM of three experiments performed in triplicate. The optical density of the band of Bcl-x_L protein expression detected by Western blotting was normalised with alpha-tubulin. Statistical comparison between groups were made using parametric Bonferroni test. Differences were considered significant if $p < 0.05$.

3. Results

3.1. DEX inhibits TRAIL-induced apoptosis

TRAIL-induced apoptosis and the effect of DEX were assessed on four different thyroid cancer cell lines. Cells were incubated as described in the Materials and methods section with either vehicle (ethanol) or 10 μM DEX. After 4 h, TRAIL (5 ng/ml) was added. Apoptosis was evaluated by cytofluorimetric analysis by PI staining of hypodiploid nuclei 24 h after TRAIL addition. Results in Fig. 1 show that TRAIL stimulated apoptosis in FRO, ARO and NPA cells, but not in WRO cells. The larger percent of apoptosis was observed in FRO cells. DEX inhibited TRAIL-induced apoptosis in FRO (−77%, $p < 0.001$) and ARO (−28%, $p < 0.05$) cells. On the basis of these results we decided to use FRO cells for the successive experiments. To investigate the mechanisms of DEX inhibition of apoptosis, the specific steroid receptor antagonist RU486 was used. In Fig. 2, TRAIL-induced apoptosis of FRO cells was assessed by caspase-3 activation and percentage of hypodiploid nuclei

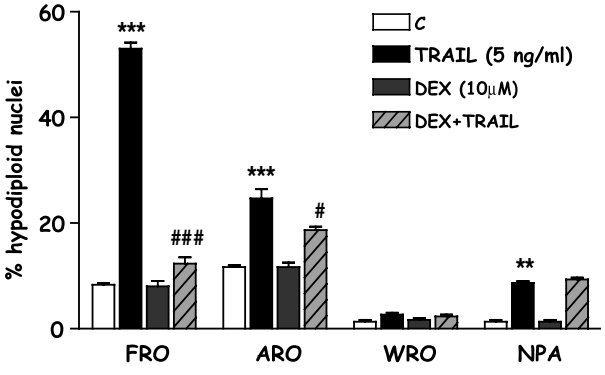

Fig. 1 – Follicular undifferentiated thyroid cancer cells (FRO), undifferentiated thyroid carcinoma cells (ARO), follicular carcinoma cells (WRO), and papillary carcinoma cells (NPA) (1×10^6 cells/well in 10-cm cell culture plates) were cultured as indicated in the Methods section. Control (C) cells were treated with vehicle (ethanol). Dexamethasone (DEX, 10 μM) was added to treated cells for 4 h before adding soluble human recombinant Superkiller TRAIL (5 ng/ml). After 24 h, apoptosis was assessed by propidium iodide (PI) staining of hypodiploid nuclei by flow cytometry. $^{***}p < 0.001$ versus C FRO and C ARO; $^{###}p < 0.001$ versus TRAIL FRO; $^{#}p < 0.05$ versus TRAIL ARO; $^{**}p < 0.01$ versus C NPA ($n = 3 \pm$ SEM).

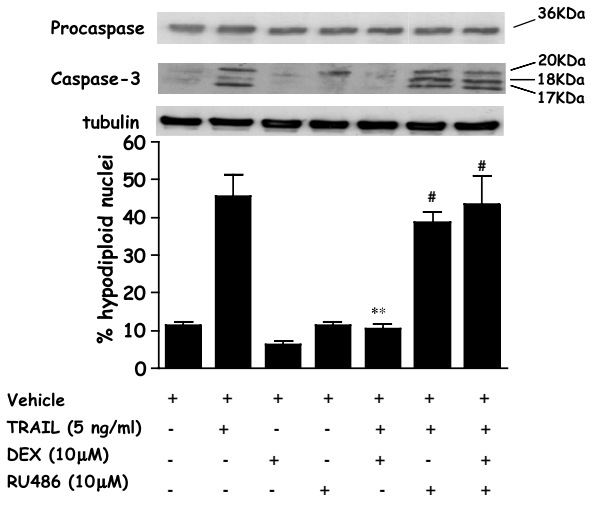

Fig. 2 – Dexamethasone inhibits TRAIL-induced apoptosis via receptor-mediated mechanisms. Follicular undifferentiated thyroid cancer cells (FRO) (1×10^6 cells/well in 10-cm cell culture plates) were cultured as indicated in the Methods section. Vehicle (ethanol), dexamethasone (DEX, 10 μM), RU486 (10 μM) or DEX (10 μM) + RU486 (10 μM) were then added to cells for 4 h before adding soluble human recombinant Superkiller TRAIL (5 ng/ml). After 6 h, caspase-3 activation was assessed by Western blotting. After 24 h, apoptosis was assessed by propidium iodide (PI) staining of hypodiploid nuclei by flow cytometry. $^{***}p < 0.001$ versus TRAIL; $^{\#}$not significant versus TRAIL ($n = 3 \pm$ SEM).

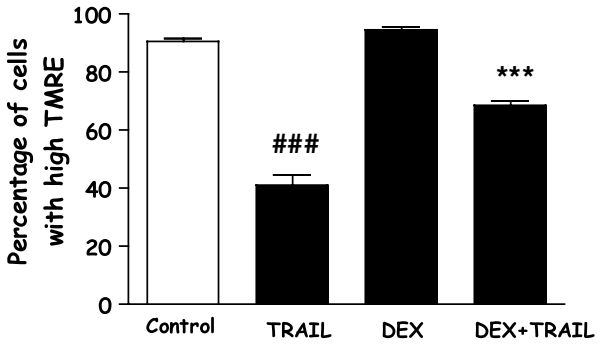

Fig. 3 – Dexamethasone inhibits TRAIL-induced mitochondrial depolarization ($\Delta\psi_m$). FRO cells were incubated for 4 h with 10 μM DEX or vehicle and for a further 24 h with 5 ng/ml TRAIL. Loss of $\Delta\psi_m$ was assessed using fluorescence-activated cell sorter (FACS) analysis of cells stained with tetramethylrhodamine ethyl ester (TMRE). The fluorescence intensity of TMRE was monitored at 582 nm (FL-2). $^{\#\#\#}p < 0.001$ versus control; $^{***}p < 0.001$ versus TRAIL ($n = 3 \pm$ SEM).

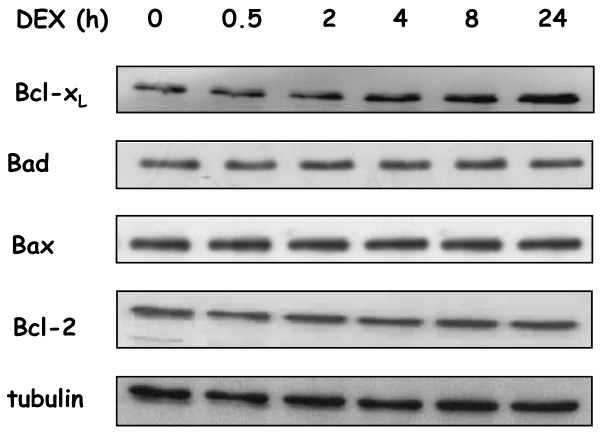

Fig. 4 – Dexamethasone stimulates Bcl-x_L expression. FRO cells were incubated with dexamethasone (10 μM) for different times (0–24 h). At the end of the incubations expression of Bcl-x_L, Bcl-2, Bax, and Bad was measured by Western blotting.

(6 and 24 h after 5 ng/ml TRAIL respectively). Again DEX (10 μM, 4 h before TRAIL) inhibited the TRAIL-induced cell death bringing back the extent of apoptosis to control (vehicle) values. The DEX-induced inhibition of apoptosis was reverted by RU486 (10 μM, added together with DEX), strongly suggesting that the DEX action is mediated by receptor activation. RU486 alone had no significant effect on TRAIL-induced apoptosis.

Next, we measured mitochondrial membrane depolarisation ($\Delta\psi_m$) as an early apoptotic marker using FACS analysis of cells stained with a fluorescent $\Delta\psi_m$ indicator, TMRE. Depolarisation of $\Delta\psi_m$ is represented by the loss of TMRE fluorescence. FRO cells were incubated for 4 h with 10 μM DEX or vehicle and for a further 24 h with 5 ng/ml TRAIL. Results in Fig. 3 show that the majority of cells in the control group had high TMRE fluorescence. Exposure to TRAIL shifted the predominant population to lower TMRE fluorescence. DEX alone had no effect, but significantly protected against TRAIL-induced loss of $\Delta\psi_m$.

3.2. *DEX stimulates the expression of Bcl-x_L*

It has been suggested that the anti-apoptotic effects of glucocorticoids may result from an increased expression of the anti-apoptotic members of Bcl family, like Bcl-x_L and Bcl-2.[2] Therefore we investigated the role of these proteins in our system. FRO cells were incubated with 10 μM DEX for different times. Expression of proteins was assessed by Western blotting at the end of incubations. Fig. 4 shows that DEX stimulated in a time-dependent fashion the expression of Bcl-x_L, but not that of Bcl-2, Bax and Bad.

3.3. *Effect of DEX on Bcl-x_L mRNA in FRO cells*

Bcl-x_L is known to be induced at a transcriptional level by dexamethasone in several systems.[12,13] Therefore, Real-time quantitative PCR was used to analyse Bcl-x_L mRNA level in FRO cells cultured for 0, 4 and 8 h in the presence or absence of DEX. Fig. 5 shows that DEX (10 μM) induced a time-dependent increase of Bcl-x_L transcript levels with peak effect at 8 h ($p < 0.01$).

3.4. *Small interfering (si) RNAs direct against Bcl-x_L inhibit expression of the protein*

In order to further investigate the role of Bcl-x_L in DEX-mediated protection from TRAIL-induced apoptosis, the technique of small interfering RNAs was utilised. FRO cells were

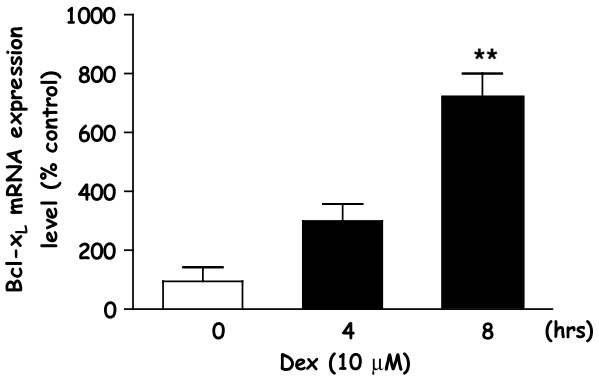

Fig. 5 – Dexametasone induces Bcl-xL mRNA expression in FRO cells. Real-time PCR analysis of Bcl-xL gene expression was performed after 4 and 8 h of incubation with 10 μM DEX. Bcl-xL mRNA levels are normalised with those of GAPDH and presented as % of the control, with the average of the controls set to 100%. $^{**}p < 0.01$ ($n = 3 \pm$ SEM).

transfected using oligofectamine with siRNAs designed against Bcl-x_L or with a scrambled siRNA, as described in the Materials and methods section. After 48 h, 10 μM DEX was added for 4 h followed by the addition of TRAIL (5 ng/ml). After a further 6 h, Bcl-x_L expression was assessed by Western blotting. Results in Fig. 6 show that basal Bcl-x_L expression was inhibited by TRAIL, but strongly stimulated by DEX. Transfection of the cells with Bcl-x_L siRNAs inhibited both basal and DEX-stimulated Bcl-x_L expression. The scrambled oligos had no effect on either basal or stimulated Bcl-x_L expression.

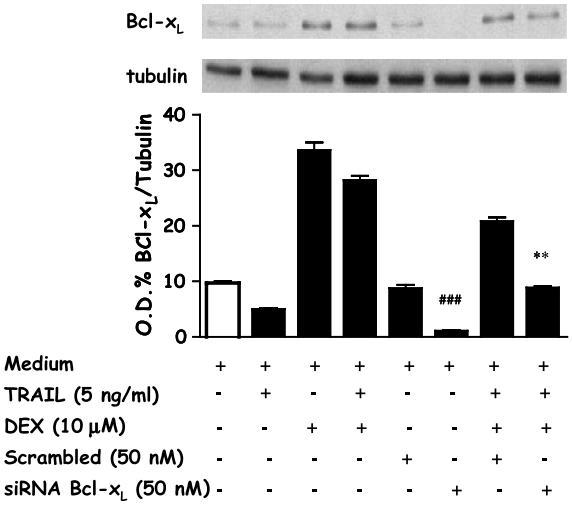

Fig. 6 – Small interference (si)RNAs against Bcl-X_L inhibit dexamethasone-induced Bcl-x_L expression. 1×10^5 FRO cells were plated in six-well plates at 30–50% confluency and transfected using oligofectamine with siRNAs against Bcl-x_L or with scrambled oligos at a final concentration of 50 nM. After 48 h Dex (10 μM) was added for 4 h before adding TRAIL (5 ng/ml). After a further 6 h, Bcl-x_L expression was assessed by Western blotting. $^{###}p < 0.001$ versus medium; $^{***}p < 0.001$ versus DEX + TRAIL ($n = 3 \pm$ SEM).

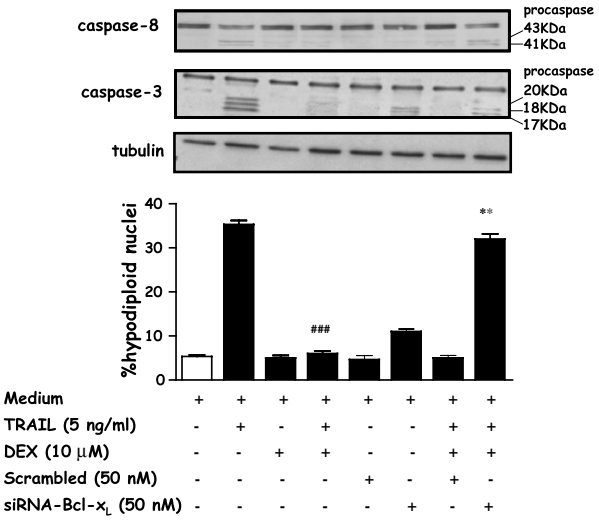

Fig. 7 – Small interference (si)RNAs against Bcl-x_L abolishes dexamethasone-induced inhibition of TRAIL-stimulated apoptosis. 1×10^5 FRO cells were plated in six-well plates at 30–50% confluency and transfected using oligofectamine with siRNAs against Bcl-x_L or with scrambled oligos at a final concentration of 50 nM. After 48 h, Dex (10 μM) was added for 4 h before adding TRAIL (5 ng/ml). After 6 h, activation of caspase-3 and caspase-8 was assessed by Western blotting. After 24 h, apoptosis was assessed by propidium iodide (PI) staining of hypodiploid nuclei by flow cytometry. $^{###}p < 0.001$ versus TRAIL; $^{***}p < 0.001$ versus DEX + TRAIL ($n = 3 \pm$ SEM).

3.5. *Small interfering (si) RNAs direct against Bcl-x_L abolish DEX-induced inhibition of TRAIL-stimulated apoptosis*

Having established that transfection with specific siRNAs efficiently down-regulated Bcl-x_L expression, the next step was to investigate the effect of Bcl-x_L knockdown on DEX-induced inhibition of apoptosis. FRO cells were transfected using oligofectamine with either siRNAs designed against Bcl-XL or with a scrambled siRNA, as in Fig. 6. After 48 h, 10 μM DEX was added for 4 h before adding TRAIL (5 ng/ml). After 6 h, activation of both caspase-3 and caspase-8 was assessed by Western blotting. After 24 h, apoptosis was assessed by PI staining of hypodiploid nuclei by flow cytometry. Results in Fig. 7 show that TRAIL stimulated apoptosis of FRO cells as measured by percent hypodiploid nuclei and caspase activation. TRAIL-induced apoptosis was again strongly inhibited by preincubation of the cells with DEX. However, transfection of the cells with siRNAs against Bcl-x_L weakly stimulated control cell apoptosis and restored apoptosis in TRAIL-stimulated cells pre-treated with DEX. The scrambled oligos had no effect suggesting that Bcl-x_L induction is required for efficient inhibition of TRAIL-induced cell death by DEX.

4. Discussion

Glucocorticoids are widely used anti-inflammatory and immunosuppressive drugs that act by both genomic[14] and non-genomic mechanisms[15] The effects of glucocorticoids

on apoptosis are also complex and vary depending upon the cell type considered. The ability of glucocorticoids to strongly promote apoptosis of lymphoid cells makes these compounds of tremendous value in the treatment of lymphoid leukaemias, lymphomas, and multiple myeloma.[16] On the other hand glucocorticoids inhibit apoptosis in a number of cells such as neutrophils,[17] osteoblasts,[18] mammary epithelial cells,[19] and hepatocytes.[20] A recent paper[8] showed that DEX is able to inhibit cisplatin-induced and TNF family ligand-induced (TNF-α, CD95L, and TRAIL) apoptosis of the cervical carcinoma cell lines P5 and HeLa. In the same paper, tumour development was induced in nude mice by subcutaneous injection of human lung carcinoma cells (P693) and while cisplatin administration inhibited tumour growth, DEX administration prevented the growth-inhibiting effect of cisplatin. Thus it appears that DEX interferes with the anti-tumour effect of chemotherapy, perhaps by protecting cancer cells from apoptosis induced by anti-neoplastic drugs. This evidence according to Herr et al.[8] raises concern about the combined use of glucocorticoids with anti-neoplastic drugs in the clinical management of cancer patients.

In the present paper we used TRAIL to induce apoptosis in four different lines of thyroid cancer cells among which follicular undifferentiated thyroid cancer cells (FRO) resulted in being the most sensitive to TRAIL induced lethal action. As discussed earlier, TRAIL appears to be a promising anti-cancer compound as it is able to kill transformed and malignant cells including most thyroid cancer cells,[4] while usually sparing normal cells. We assessed TRAIL-induced apoptosis by measuring the percent of hypodiploid nuclei (PI staining), the activation of caspase-3 and -8, and the mitochondrial membrane potential ($\Delta\psi_m$). By all techniques, DEX inhibited the TRAIL-induced apoptotic changes in FRO cells. In addition, the protective action by DEX was prevented by the steroid receptor antagonist RU486 suggesting that DEX was acting through receptor activation and modulation of gene expression.

The steroid induced inhibition of apoptosis of thyroid cells may explain the occasional clinical observations of excessive thyroid proliferation in patients with Cushing's disease. In fact, thyroid nodular disease,[21] adenoma,[22] and papillary carcinoma[23] have all been described in Cushing patients. One possible explanation is that the relative proliferation of thyroid tissues may be a consequence of a low rate of spontaneous apoptosis due to the high blood cortisol levels of these patients.

The mechanisms of apoptosis regulation by GC have been widely investigated and recently reviewed.[7,16] Both gene *trans*-activation and *trans*-repression of GR target genes appear to be involved in the GC modulation of apoptosis. There is a general consensus that the expression levels of the proteins of Bcl-2 superfamily correlate with cell sensitivity to GC-induced apoptosis. Low level of expression of Bcl-2 makes leukaemia cells sensitive to GC-induced death.[24] Bcl-2 overexpression inhibits DEX-induced apoptosis in lymphoma cells.[25] It has also been shown that DEX inhibits apoptosis by transcriptionally activating Bcl-2 and/or Bcl-x_L in hepatocytes,[20] mammary epithelial cells,[26] fibrosarcoma cells,[12] gastric cancer cells.[27] In FRO cells a time-dependent increase of Bcl-x_L was observed after DEX treatment. The steroid drug had no effect on the expression of Bad, Bax, and Bcl-2 proteins. In addition, Real-time quantitative PCR experiments showed a time-dependent increase of Bcl-x_L transcript levels by DEX suggesting that the steroid drug modulates Bcl-xL gene expression in FRO cells at the transcriptional level. The results of the experiments with siRNAs direct against Bcl-x_L confirmed the hypothesis that Bcl-x_L expression is required for protection from TRAIL-induced apoptosis in DEX-treated FRO cells. Finally, the expression of TRAIL receptors appears not to be involved in this system as no effect was observed on TRAIL receptor expression levels by DEX in the examined thyroid cancer cell lines (data not shown).

In summary, we have shown that DEX, a potent glucocorticoid agent, protects thyroid cancer cells from apoptosis induced by TRAIL, confirming results obtained in other solid tumours.[8] DEX acts through receptor activation and up-regulation of the expression of the anti-apoptotic protein Bcl-x_L. We cannot exclude that other steroid-regulated genes may contribute to the DEX protective action. It has been recently shown that the serine/threonine survival kinase gene, *sgk-1*, is involved in the protection from apoptosis by glucocorticoids in human breast cancer cells.[28] Experiments are in progress to ascertain the role of this latter gene in glucocorticoid inhibition of apoptosis in thyroid carcinoma cells.

While the majority of papillary, follicular and medullary thyroid cancers can be successfully treated with surgery and radioactive iodine, anaplastic or undifferentiated thyroid carcinomas are resistant to these traditional therapies and remain a devastating disease. Salvage chemotherapy and radiation, alone and in combination, have been the mainstay of palliative treatment for undifferentiated thyroid cancers. For example, approximately 30% of patients achieve a partial remission with doxorubicin[29] and the addition of cisplatin appears to be more active than doxorubicin alone.[30] Combination chemotherapy and radiation is currently gaining favour.[31,32] Because glucocorticoids are often administered as premedications for therapies used for anaplastic thyroid cancers, the findings presented here with the FRO cell line raise the concern that mechanisms downstream of GR activation may decrease apoptosis and inhibit the maximal therapeutic benefit of chemotherapy in these cancers. In addition, the finding that increased Bcl-$x_L$expression is anti-apoptotic with respect to TRAIL-mediated killing in FRO cells suggests that Bcl-x_L expression may be a useful predictive marker in patients with anaplastic thyroid cancers receiving experimental DR4 and DR5 targeted therapies.

Conflict of interest statement

None declared.

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