Supplementary Materials and Methods

Reagents and antibodies. DMEM, Dyna M280 streptavidin beads, RPMI 1640, sodium pyruvate and TMRE were obtained from Invitrogen (Paisley, UK). FITC conjugated Annexin V was obtained from Bender Medsystems (Vienna, Austria). PE conjugated anti-human TRAIL-R1 /-R2 and PE Mouse IgG1, κ Isotope Control were obtained from EBioscience (San Diego, USA). Goat sera, Digitonin, FCCP, rotenone and 2DG were obtained from Sigma-Aldrich (Poole, UK). Z-VAD.fmk was obtained from MP Biomedicals (Illkirch, France) and BD Cell-Tak ™ was obtained from BD Biosciences (Lutterworth, UK). Human recombinant TRAIL and caspase-8 antibodies were made in house (1).

Primary cell experiments. Primary mantle cell lymphoma cells were obtained from patients during routine diagnosis at the Leicester Royal Infirmary (Samples were obtained with patient consent and local ethical committee approval) and were purified as described previously (2).

Induction and analysis of apoptosis. Z138 cells were harvested at a concentration of 1 x 10^6 cells/ml and treated with various concentrations of TRAIL (0 – 1000 ng/ml), ABT-737 (0 – 1000 nM) or 2DG (5 mM) as indicated. For the ionizing radiation experiments, Z138 cells were seeded up to 48 h prior to treatment and harvested at a concentration of 1 x 10^6 cells/ml. Cells were then irradiated using a Pantak X-ray unit at a dose of 35 GY and assessed for apoptosis induction at the indicated times post-radiation. Phosphatidyl serine (PS) exposure and loss of mitochondrial membrane potential ($\Delta\psi M$) were assessed after treatment as previously described (2, 3). Caspase-8, -3 and PARP cleavage was examined on cell pellets taken from treated cells by western blotting as previously described (4).

TRAIL Receptor Expression. Cell surface expression of TRAIL receptors was determined as previously described (1, 2). Cells (1 x 10^6) were resuspended and incubated in 10% goat sera for 5 min at room temperature, before further incubating with receptor–specific PE conjugated antibodies for 1 h at 4ºC. The cells were washed twice by centrifugation/resuspension with 1 ml ice cold PBS and resuspended in 500 µl PBS before analysis with a FACSCalibur flow cytometer and CellQuest Pro™ Software. Antibody binding was assessed using a single-parameter histogram with 'cell count' on the y-axis and a log scale of the FL2-channel fluorescence on the x-axis.

TRAIL DISC analysis. Biotinylated TRAIL (bTRAIL) was prepared and used to affinity purify the TRAIL DISC as previously described (1, 4). Briefly, Z138 cells (60 x 10^6) pre-treated with bTRAIL (500 ng/ml) for 1 h on ice were incubated at 37 ºC for the indicated times, then washed three times with ice cold PBS and lysed on ice for 30 min. Lysates were cleared by centrifugation (15,000 x g for 30 min at 4 ºC) and bTRAIL complexes captured overnight at 4 ºC using Dyna M280 streptavidin

beads. TRAIL DISC proteins were eluted from beads by boiling in SDS–PAGE sample buffer and analysed by immunoblotting. Isolated DISC activity was assayed fluorimetrically using ac.IETD.afc as previously described (5).

Cytochrome c release. Cytochrome *c* release was determined as previously described (6, 7). Briefly, 10 x10^6 cells were washed in ice cold PBS and resuspended in cytochrome *c* lysis buffer (250 mM sucrose, 20 mM HEPES, 5 mM $MgCl_2$ and 10 mM KCL, pH 7.4) containing 0.05 % digitonin and protease inhibitors. Cells were left on ice for 10 min followed by centrifugation at 13,000 g for 3 min, 4 ºC. Supernatant and pellets were separated and analysed for cytochrome *c* by western blotting.

Glycosidase treatment

Endoglycosidases can be used to determine whether or not a protein contains N-linked glycans. PGNase F (cleaves between GlcNac and Asn and is specific for complex glycan structures whereas Endo H cleaves chitobiose core high mannose and hybrid-type glycosylated proteins. Therefore glycosylation of TRAIL-R1 was determined by digestion of purified TRAIL DISC using Endoglycosidase H (Endo H) and N-Glycosidase F (PNGase F) according to the manufacturer's protocol. Briefly, isolated TRAIL DISCs were incubated with 1000 U of either EndoH or PNGase F for 2 h @ 37 ºC. After incubation, samples were diluted with SDS-PAGE sample buffer, heated to 95 ºC for 5 minutes and analysed by immunoblotting as described in the Materials and Methods.

***N*-Acetylglucosamine**

Treatment of glucose-free Z138 cells to test for glycosylation efficiency was carried out by incubating cells with 5 mM *N*-Acetylglucosamine (NAG) for 2h followed by TRAIL (0-1000 ng/ml) for 4 h in the same media. Apoptosis was then assessed as described previously.

Transcriptional Analysis

Transcriptional analysis of glucose-containing and glucose-free Z138 cells was performed in collaboration with the Systems Toxicology Group, MRC Toxicology Unit as previously described (8). Briefly, two colour microarrays were carried out using a full-genome human oligo array (70 mer) with exon centred probes (Human Exonic Evidence Based Oligonucleotide) (Invitrogen). A total of 49, 000 probes were covered, including various spike-ins, non-coding RNA and control spots, and were printed in-house using an Inkjet style ArrayJet Printer (AJ120 Ultra-Marathon) over two aldehyde slides (Genetix). Total RNA was extracted from glucose-containing or glucose-free cells using a miRNeasy Mini Kit (Qiagen), according to the manufacturers' protocol, and labelled using the Low Input Quick AMP Labelling Kit (Agilent). Labelled cRNA was purified using an RNeasy MiniElute Clean-up Kit (Qiagen) and dye incorporation assessed using a nanodrop ND-1000 UV Spectrophotometer (Nanodrop Technologies). cRNA was fragmented using fragmentation reagent

(Ambion) and samples hybridised on to the microarray slides overnight at 42 ºC. Slides were scanned using an Axon 4200A scanner with Genepix 6.0 software. For each analysis three biological repeats were used, each with two technical repeats (reverse labelling).

Translational Analysis

Polysome profiles were measured as previously described (9). Briefly, cyclohexamide (0.1 mg/ml) was added to control and 2DG treated cells followed by cell lysis. The cell lysates were then separated onto 10-60 % sucrose density gradients and centrifuged at 248000 g for 2 h at 4 °C. Gradients were fractionated with the eluate measured at absorbance 254 nm before collection.

Supplementary References

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