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Investigation of TRAIL Resistance in Lung Cancer Cell Lines

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Introduction

TNF-related apoptosis-inducing ligand (TRAIL) is a important protein expressed by Natural Killer cells within the immune system.

It induces apoptosis preferentially in cancer cells and is a potential therapeutic target

Many tumours are TRAIL-resistant, and TRAIL-resistance emerges readily during therapy.

TRAIL-sensitisors may overcome both existing, and emerging TRAIL insensitivity (see Fig 1)

Non-proliferative quiescence cancer stem cell-like cells are TRAIL-resistant in some tumour models (Cross, NA. Unpublished observations).

Translational reprogramming agents such is EIF2 inhibitors may overcome the quiescent phenotype and sensitise cells to (Schewe & Aguirre-Ghiso, 2009).

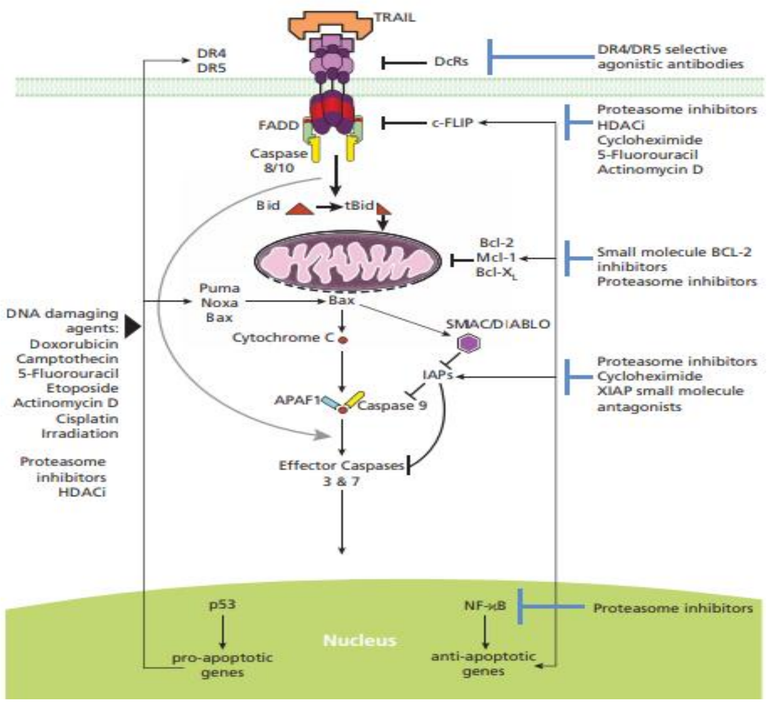

Figure 1. Known TRAIL-sensitisers and mechanisms of action (Cross and Sayers, 2014)

Aims and Hypothesis

The hypothesis of this study was that quiescent cells isolated from lung cancer could be translationally reprogrammed to bring them out of a resistant state.

Aims:

1) Isolate a quiescent phenotype within lung cancer cell lines.

2) Induce translational reprogramming using drug Salubrinal and re-sensitize the population.

Methods

Non-proliferating cells from lung cancer cells were distinguished using lipophilic membrane PKH67 dye which is lost on cell proliferation and flow cytometry to identify PKH67^{Hi} non-proliferating cells.

Apoptosis was assessed in response to TRAIL at 24 and 72 hours using Propidium Iodide and Hoechst 33342 staining.

Cells were re-challenged with to establish a TRAIL resistant (TRAIL^{R}) population vs. the parent line

•TRAIL^{R} cells were treated with Salubrinal (10/25µm) in combination with TRAIL cells to assess the effects of translational reprogramming on acquired TRAIL^{R}

References Schewe, D& Aguirre-Ghiso (2009). Inhibition of eIF2 Dephosphorylation Maximizes Bortezomib Efficiency and Eliminates Quiescent Multiple Myeloma Cells Surviving Proteasome Inhibitor Therapy. *Cancer Research, 69*(4), 1545-1552. -

Results

A549 does not contain a quiescent population, and all PKH67^{Hi} cells were associated with spontaneous cell death (Fig 2)

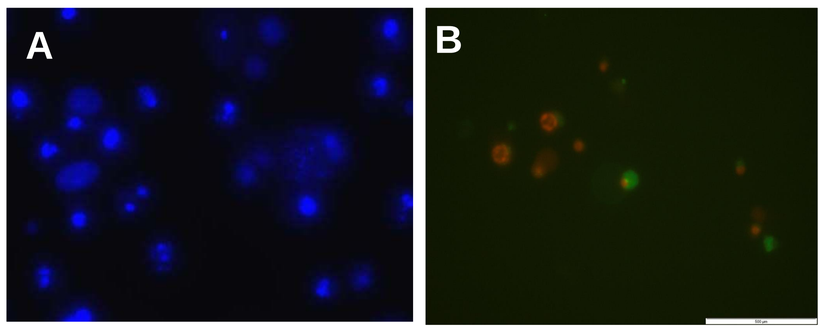

Figure 2. (**A**) A549 cells stained with Hoechst 33342 and stimulated with 25ng/mL of TRAIL at 24 hours imaged using fluorescent microscopy to show apoptosis. **(B)** Images show PKH67^{Hi} cells undergoing apoptosis when exposed to 25ng/mL of TRAIL at 72 hours in cell line A549 at 20x magnification.

Identification of quiescent cells

Flow cytometry was used to identify a population of cells which retained PKH67 over a 12 day period. In the A549 cell lines, there was no high presence of PKH67 high cells.

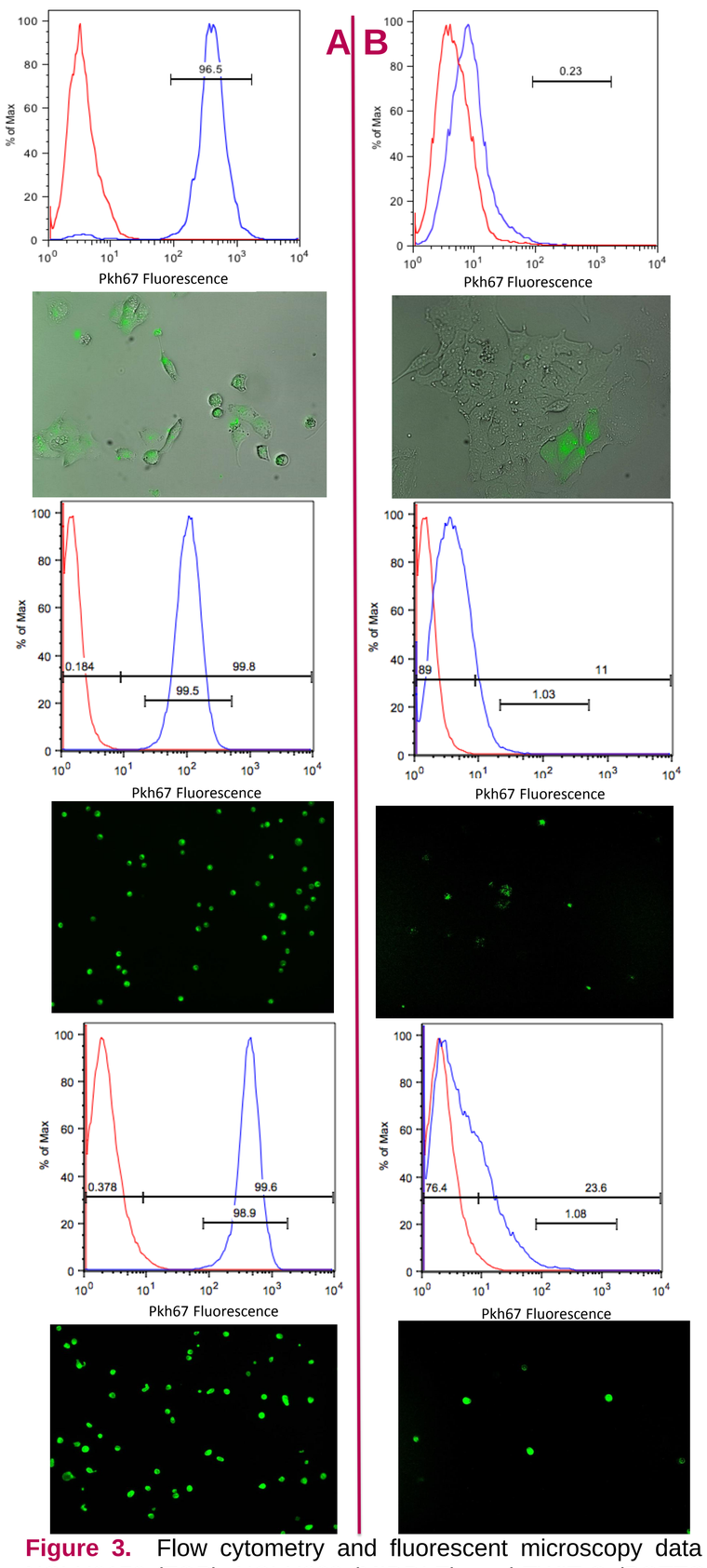

Figure 3. Flow cytometry and fluorescent microscopy data of NCI-H2170 (TOP), NCI-H838 (MIDDLE), and TWIT-Q (BOTTOM) cells. Column (A) represents PKH67 stain populations compared to the parent strain taken at day 1 and column (B) represents the same cells taken at day 12. Microscopy images taken using the FITC camera of the cells at the same time as flow cytometry are displayed underneath the corresponding flow cytometry data.

Investigation of Translational reprogramming agent Salubrinal on TRAIL^{R} Populations

The TRAIL sensitivity of 4 cell lines was determined (fig 4) and subsequently, TRAIL^{R} populations isolated after persistent TRAIL-treatment by culture of surviving cells after TRAIL treatment. TRAIL^{R} populations were confirmed to be less sensitive to TRAIL than parental populations (fig 5). TRAIL did not synergistically enhance TRAIL responses in TRAILR cells (Fig 6).

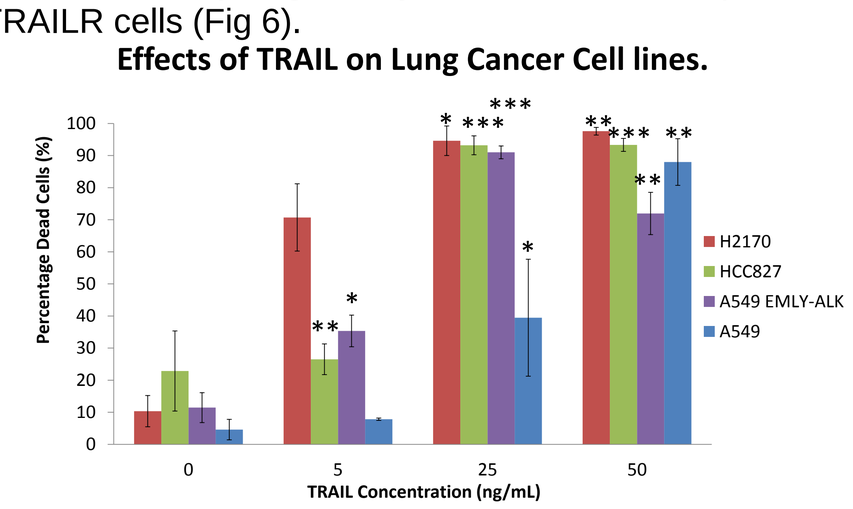

Figure 4. The percent apoptosis in lung cancer cell lines when exposed to TRAIL at 0150ng/ml for 24hours. Comparison of treated vs. untreated for each cell line is shown and assessed by Kruskal-Wallis multiple comparisons test (p<0.05=* P<0.01=** P<0.001).

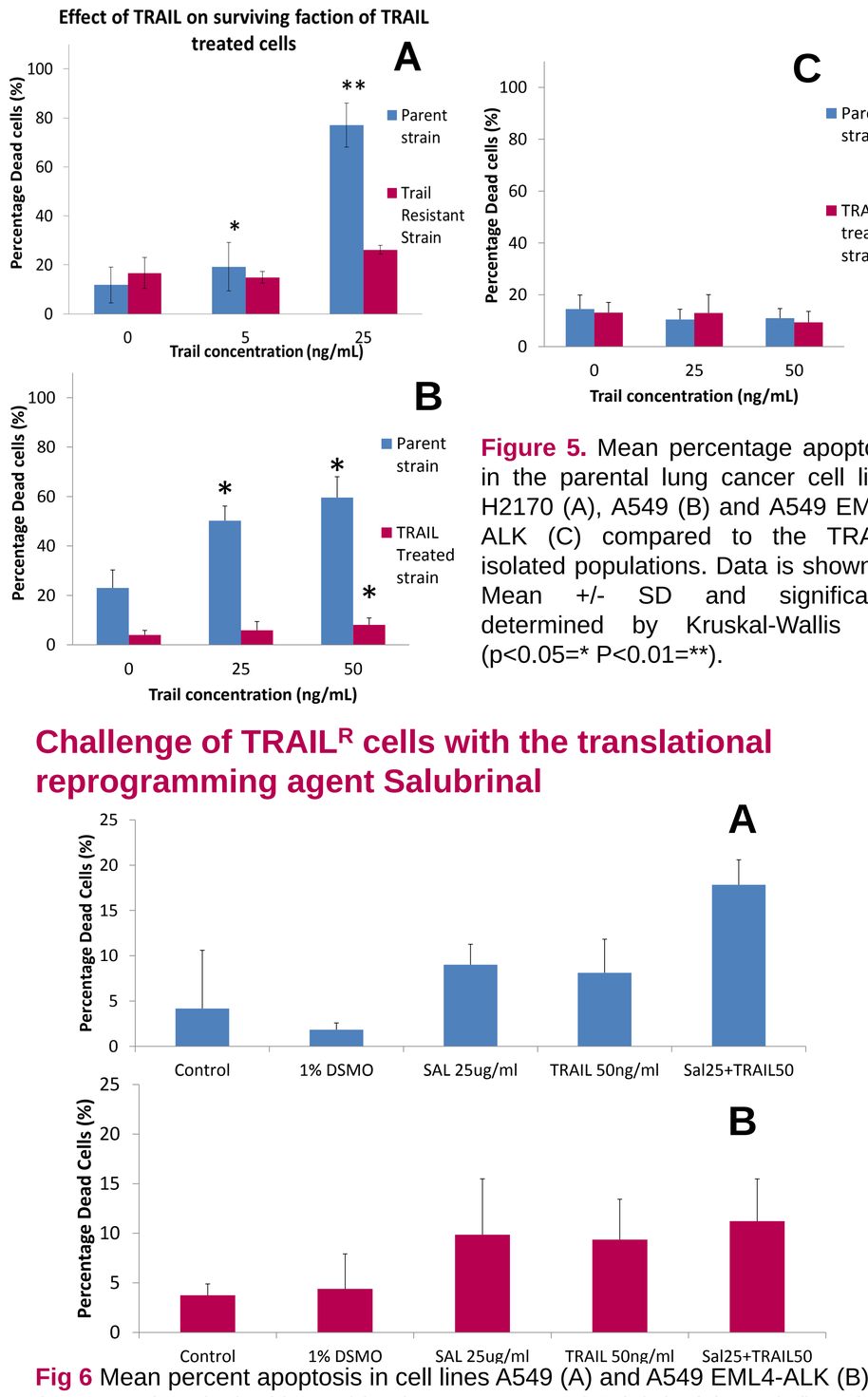

Figure 5. Mean percentage apoptosis in the parental lung cancer cell lines H2170 (A), A549 (B) and A549 EML4-ALK (C) compared to the TRAIL^{R} isolated populations. Data is shown as Mean +/- SD and significance determined by Kruskal-Wallis test (p<0.05=* P<0.01=**).

Challenge of TRAIL^{R} cells with the translational reprogramming agent Salubrinal

Fig 6 Mean percent apoptosis in cell lines A549 (A) and A549 EML4-ALK (B) (ALK translocation) with combination treatments of Salubrinal (25µg/ml) and TRAIL (50ng/ml).

Conclusions and future work.

The cell lines A549, and NCI-H2170 do not contain a quiescent population. However more recent work has successfully isolated quiescent cells from other lung cancer cell lines (Fig 3).

TRAIL-sensitive cell lines can readily be made TRAIL^{R} by persistent treatment with sub-toxic doses of TRAIL, mirroring clinical findings.

TRAIL^{R} cells could not be sensitised to TRAIL by Salubrinal

Ongoing work is aimed at assessing gene expression changes in TRAIL^{R} cells and assessing TRAIL resistance in quiescent cells in the new TWIT-Q cell line.