Investigation of TRAIL resistance in lung cancer cell lines

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Introduction
TNF-related apoptosis-inducing ligand (TRAIL) is an 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 quiescent cancer stem cell-like cells are TRAIL-resistant in some tumour models (Cross, NA. Unpublished observations). Translational reprogramming agents such as EIF2 inhibitors may overcome the quiescent phenotype and sensitize cells to (Schewe & Aguirre-Ghiso, 2009).

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 Salubralin 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* 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 (TRAILR) population vs. the parent line.

TRAILR cells were treated with Salubralin (10/25µm) in combination with TRAIL cells to assess the effects of translational reprogramming on acquired TRAILR

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

Results
A549 does not contain a quiescent population, and all PKH67* cells were associated with spontaneous cell death (Fig 2).

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.

Investigation of Translational reprogramming agent Salubralin on TRAILR Populations
The TRAIL sensitivity of 4 cell lines was determined (fig 4) and subsequently, TRAILR populations isolated after persistent TRAIL-treatment by culture of surviving cells after TRAIL treatment. TRAILR populations were confirmed to be less sensitive to TRAIL than parental populations (fig 5). TRAILR cell lines were sensitised by Salubralin (Fig 6).

Conclusion and future work.
The cell lines A549, and NC1H2170 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 TRAILR by persistent treatment with sub-toxic doses of TRAIL, mirroring clinical findings. TRAILR cells could not be sensitised to TRAIL by Salubralin. Ongoing work is aimed at assessing gene expression changes in TRAILR cells and assessing TRAIL resistance in quiescent cells in the new TWIT-Q cell line.

References