An arrayed genome wide CRISPR/Cas9 screen for activators of dendritic cells

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Introduction

Tumour immunogenicity is defined as the ability of the tumour to induce an immune response (Figure 1). Immunogenicity is determined by two main components: **antigenicity** (presence of neoantigens that can be presented on MHC class I and recognised by cytotoxic T cells) and **adjuvanticity** (presence of signals that will activate antigen presenting cells, enabling them to stimulate tumour-specific T cells).

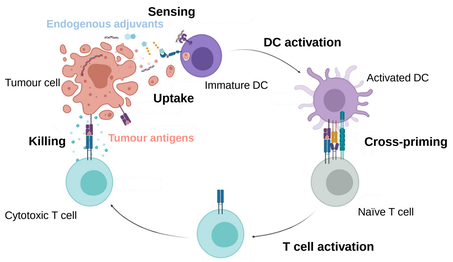


Fig 1. Schematic depiction of the cancer immune cycle

The adjuvants produced by cancer cells result from dysregulation of cancer-intrinsic pathways. Upon sensing of the cancer cell-derived adjuvants, DCs undergo a maturation program during which they increase their capacity to cross-present tumour-derived antigens and upregulate cell surface molecules required for co-stimulation of T cell (e.g. CD83 and CD86). Both, presentation of neoantigens on MHC class I and surface expression of co-stimulatory molecules are required to licence tumour-specific cytotoxic T cells, leading to anti-tumour immune responses. Therefore, to fully understand how anti-tumour immune responses are initiated, we need to obtain a more complete picture of the tumour-derived DAMPs involved.

To identify genes and pathways relevant for cancer cell adjuvanticity, we set up a high-throughput cancer cell-DC co-culture assay to monitor DC activation in response to perturbations in cancer cells.

The dendritic cell model

MutuDC is a murine DC line derived from spleen tumours formed in CD11c:SV40LgT-transgenic C57BL/6 mice (Fuertes Marraco et al., 2012). MutuDCs respond to stimulation with synthetic adjuvants such as Poly(I:C) and prostaglandin E2 (PGE2), upregulating different combinations of CD86, CD83, CD80 and CD40 (Figure 2)

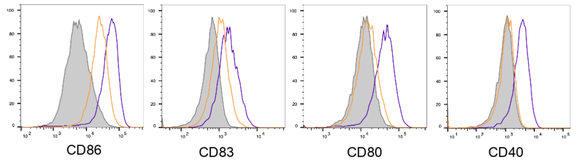


Fig 2. Membrane staining intensity of CD86, CD83, CD80 and CD40. MutuDCs were left untreated (solid grey) or treated with PGE2 (orange line) or Poly(I:C) (purple line) for 5 hours.

The cancer cell model

MutuDCs respond to co-culture with other cell types, upregulating maturation markers to different levels. We co-cultured MutuDCs with several human cancer cell lines and measured levels of maturation markers after co-culture using FACS (Figure 3).

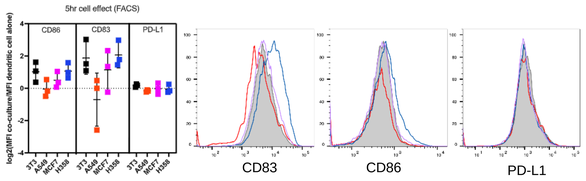


Fig 3. Membrane staining intensity of CD86, CD83 and PD-L1. MutuDCs were left untreated (solid grey) or co-cultured with A549 (orange line), MCF-7 (lavender line) or H358 (blue line) for 5 hours.

We selected the lung adenocarcinoma cell line A549 as it did not activate MutuDCs in co-culture in the absence of perturbations. A549 is available at AstraZeneca with stable integration of the tightly doxycycline controlled inducible spCas9 transgene ODInCas9 (Lundin et al, 2020).

The co-culture assay

We culture the cancer cells, exposing them to treatments if necessary. We then add MutuDC at a DC:cancer cell ratio of 2:1. We incubate the co-cultures under different conditions to induce peturbations. We then either collect the DCs and stain for flow cytometry, or fix the co-cultures and stain for imaging (Figure 4). If the cancer cells produce adjuvants, we detect a higher level of membrane CD83 and CD86.

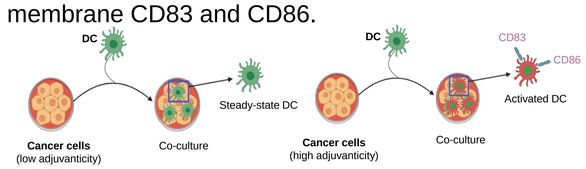
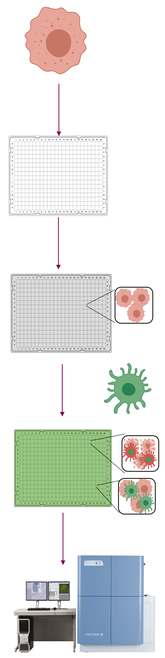


Fig 4. Schematic of the co-culture and the output depending on whether cancer cells produce low (left) or high (right) amounts of adjuvants.

A genome wide CRISPR/Cas9 screen



1) ODInCas9 A549 cells are expanded and Cas9 expression is induced

2) An arrayed genome-wide sgRNA library is acoustically dispensed to assay plates

3) A549 cells are reverse transfected with the library and incubated 48 hours

4) MutuDCs are plated and co-cultures are incubated for 16 hours

5) Co-cultures are fixed and stained for CD83 and CD86

6) Co-cultures are imaged using a high throughput confocal system (Yokogawa CellVoyager 7000)

Results from the screen

We analysed more than 18,000 unique genes and selected 320 candidate genes to take forward (Figure 5, left). The list of candidate genes was enriched for several complexes and pathways, for example the Eukaryotic translation initiation factor 4F complex (Figure 5, right).

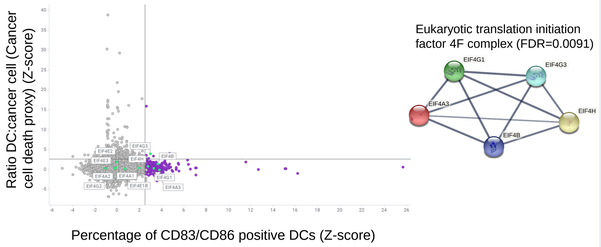


Fig 5. Left: scatter plot of screen results, candidate genes in purple and Eukaryotic translation initiation factor 4F complex genes in green. Right: String network of the enriched complex.

We repeated the co-culture assay using sgRNAs for the 320 candidate genes and selected 12 genes which showed a reproducible and consistent effect on DC activation across three replicate assays. Included in this list is EIF4A3, a member of the complex above. We have also identified other genes which have previously been found to be associated with cancer cell proliferation, invasiveness, potential for metastasis and prognosis in different types of cancer.

Future direction

We will study the function of the genes selected using a variety of methods such as:

- Validation in other cancer cell lines.
- Identification of the receptors or downstream signalling pathways in the DCs.
- Transcriptomics and/or proteomics analysis of cancer cells and DCs in co-culture.
- Validation using a human DC model, derived from induced pluripotent stem cells.