Flow cytometric phenotyping of diverse human cancer cell lines for immunological biomarkers expression

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Introduction

- Cancer immunoediting is a process that cancer cells undergo to evade the immune system (Figure 1) (Swann & Smyth, 2007).
- To eliminate nascent tumours, death receptors (DR) 4/5 and Fas respond to tumour necrosis factor-related apoptosis-inducing ligand and Fas Ligand, respectively, to induce cancer cell apoptosis.
- To escape immune-mediated killing, cancer cells can reduce the expression of death receptors (Shin *et al.,* 2001) and major histocompatibility complex (MHC) class I, as well as express co-inhibitory molecules including programmed death ligand 1 (PD-L1) (Escors *et al.,* 2018).
- Tumour escape is one of the hallmarks of cancer and it is necessary for tumour progression (Hanahan & Weinberg, 2011).
- By targeting these immune markers alone or in combination could potentially increase cancer cell death and improve drug efficacy.

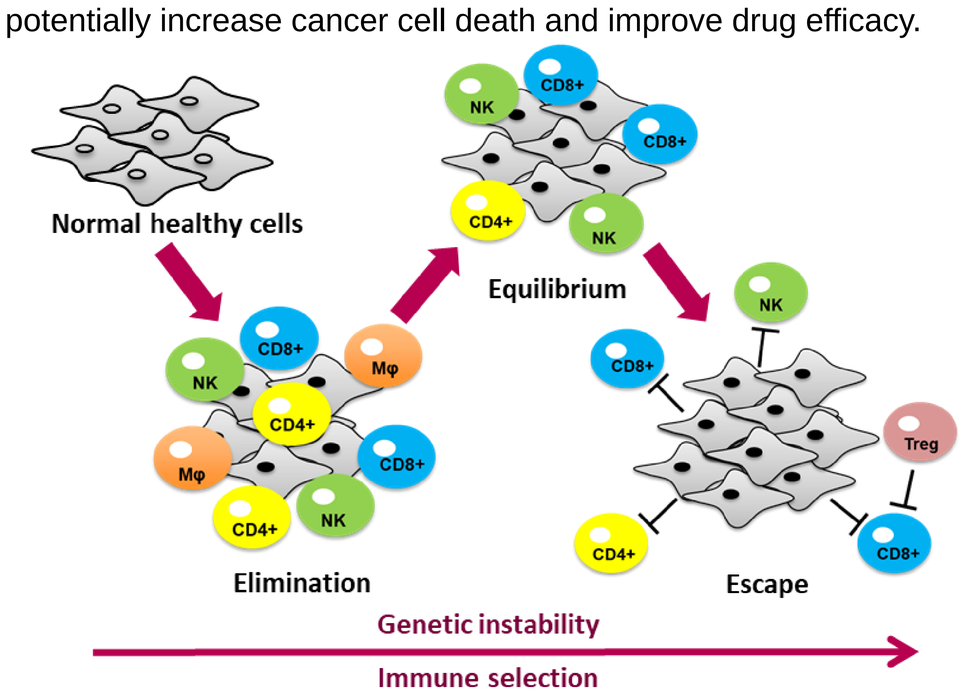

Figure 1: The process of Cancer Immunoediting – Elimination, Equilibrium and Escape. In the elimination phase, immune cells can recognise and eliminate nascent tumours, but some cancer cells avoid immune destruction and enter dynamic equilibrium with immune cells. Here, cancer cells develop increased genetic instability and undergo immune selection whereby the immune cells select for the cancer cells with the ability to evade the immune systems defences leading to immune escape and tumour progression.

Aims and Hypothesis

- This research aimed to determine the cell surface expression of immune markers including PD-L1, MHC class I, DR 4/5 and Fas in diverse human cancer cell lines.
- It was hypothesised that human breast, prostate and colorectal cancer cell lines would express PD-L1, MHC class I, DR 4/5 and Fas.

Methods

Cell culture Human cancer cell lines were cultured in DMEM (SW620 and SW480) or RPMI (MDA-MB-231, MCF-7, PC3 and LNCaP) media supplemented with 10% FBS and 1% penicillin-streptomycin.

Cell surface staining Human cancer cell lines (5 x 10^5) were stained with fluorescently labelled anti-human PD-L1, HLA-ABC, DR4/5 and Fas antibodies and their matched isotype controls. Cell surface expression was assessed using flow cytometry and data was analysed using FlowJo software.

Results

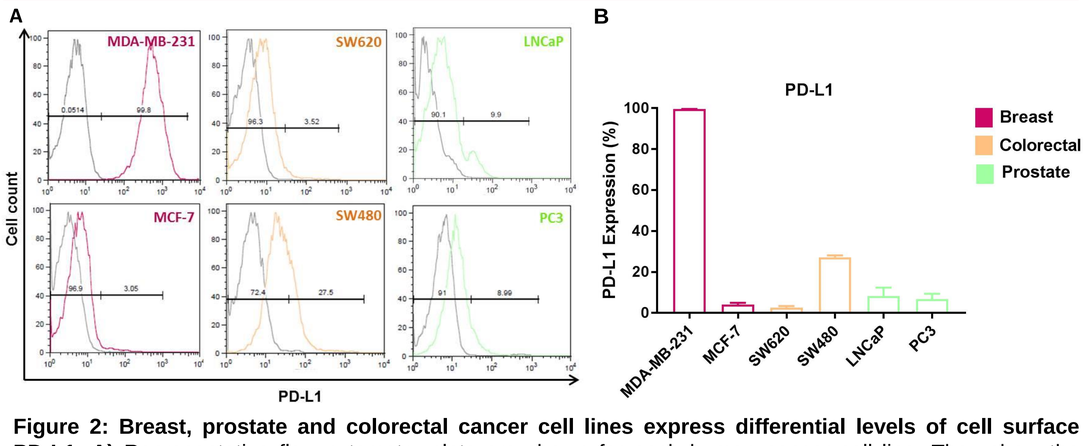

Figure 2: Breast, prostate and colorectal cancer cell lines express differential levels of cell surface PD-L1. A) Representative flow cytometry plots are shown for each human cancer cell line. They show the difference between the isotype control (grey) and the PD-L1 positive population. **B)** Breast cancer cell line MDA-MB-231 express high levels of PD-L1. Breast (**MCF-7**), prostate (**LNCaP and PC3**) and colorectal (**SW620 and SW480**) cancer cell lines express low levels of PD-L1. Data is presented as mean ± SD, n=3.

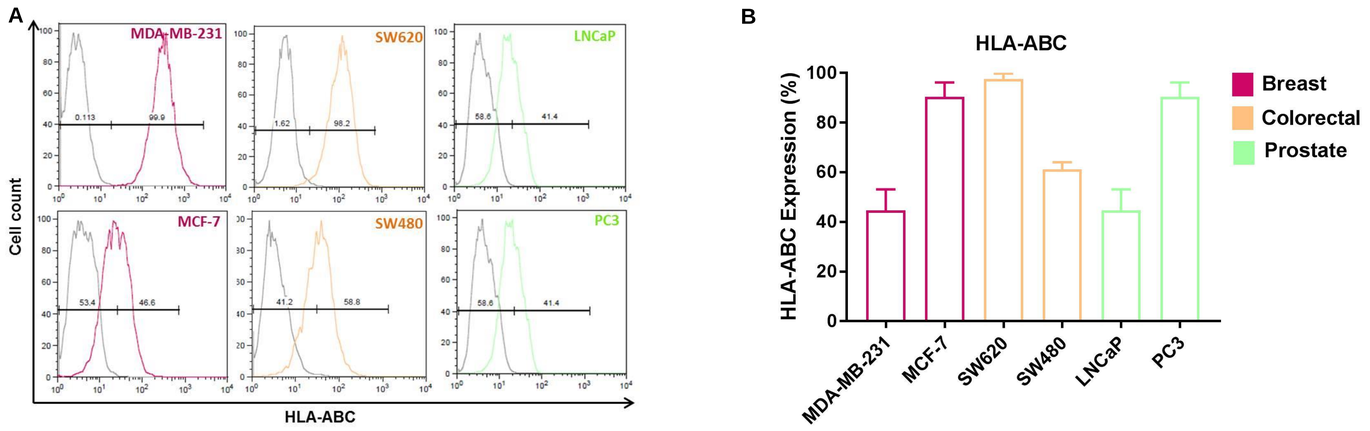

Figure 3: Breast, prostate and colorectal cancer cell lines express differential levels of cell surface MHC class I. A) Representative flow cytometry plots are shown for each human cancer cell line. They show the difference between the isotype control (grey) and the MHC class I (HLA-ABC) positive population. **B)** Breast (**MDA-MB-231 and MCF-7**), prostate (**LNCaP and PC3**) and colorectal (**SW620 and SW480**) cancer cell lines express moderate to high levels of MHC class I. Data is presented as mean ± SD, n=3.

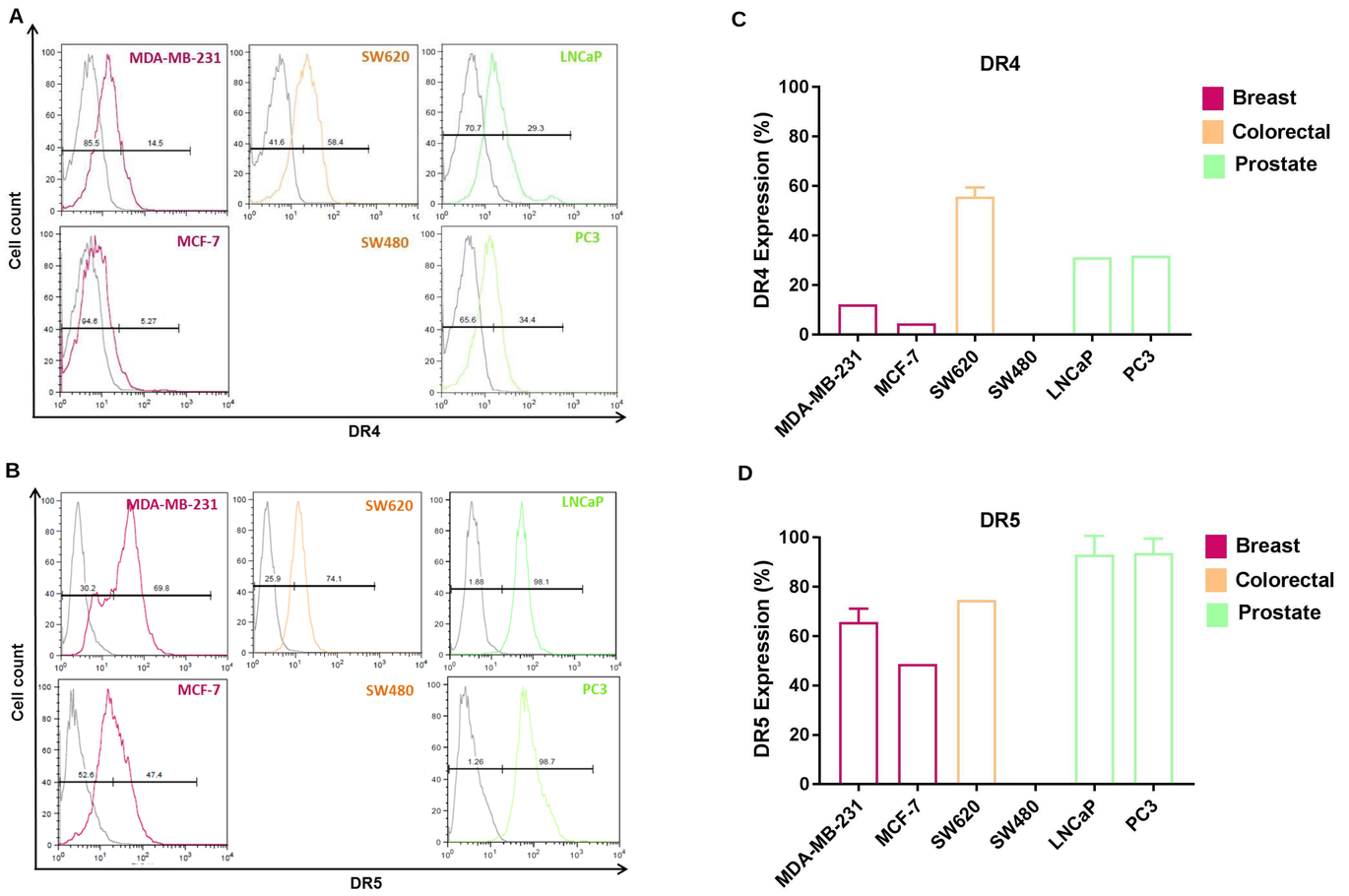

Figure 4: Breast, prostate and colorectal cancer cell lines express differential levels of cell surface DR4 and DR5. Representative flow cytometry plots are shown for each human cancer cell line. They show the difference between the isotype control (grey) and the DR4 **(A)** and DR5 **(B)** positive population. **(C)** Breast (**MDA-MB-231 and MCF-7**), prostate (**LNCaP and PC3**) and colorectal (**SW620 andSW480**) cancer cell lines have low to moderate expression of DR4. **D)** Breast (**MDA-MB-231**), prostate (**LNCaP and PC3**) and colorectal (**SW620 and SW480**) cancer cell lines express high levels of DR5, only MCF-7 displayed moderate expression of DR5. Data is presented as mean ± SD, n=2.

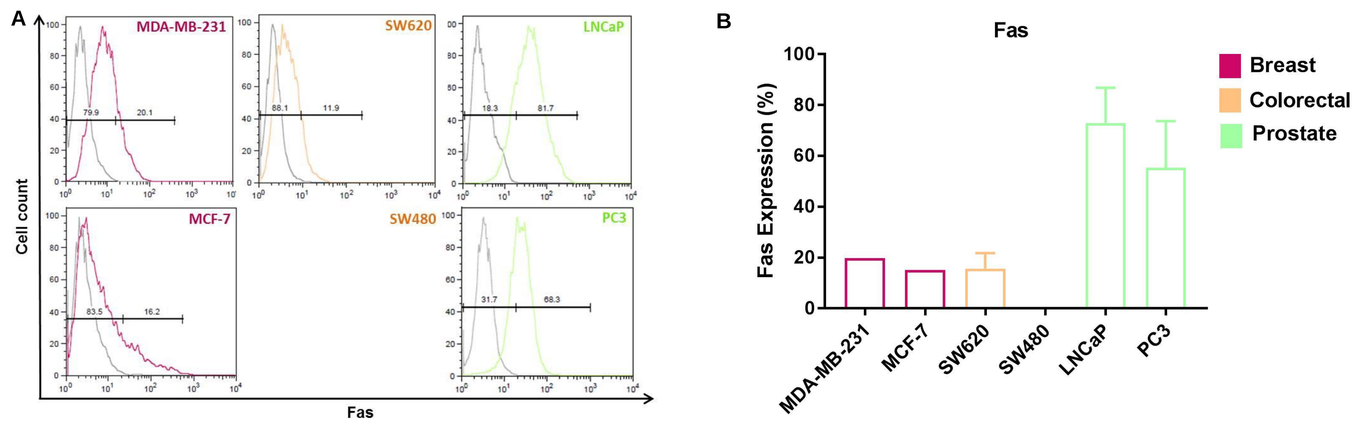

Figure 5: Breast, prostate and colorectal cancer cell lines express differential levels of cell surface Fas. A) Representative flow cytometry plots are shown for each human cancer cell line. They show the difference between the isotype control (grey) and the Fas positive population. **B)** Breast (**MDA-MB-231 and MCF-7**) and colorectal (**SW620 and SW480**) cancer cell lines express low levels of Fas whereas prostate (**LNCaP and PC3**) cancer cell lines express moderate to high levels of Fas. Data is presented as mean ± SD, n=2.

Conclusion and Future Direction

Utilising flow cytometric analysis on breast, prostate and colorectal cancer cell lines, we have found differential expression of immune markers depending on the cancer type. These findings provide a platform for future work that will entail siRNA knockdown of PD-L1 to determine the tumour-intrinsic role of this ligand, in addition to combination therapies including death receptor agonists and chemotherapeutic agents in 2D and 3D cell culture.

References

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