A powerful approach to uncover immune checkpoint receptors using human plasma membrane protein arrays (PMPA)

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BACKGROUND

Cancer cells can avoid triggering an immune response by 'switching off' T-cells through inhibitory ligand-receptor interactions at immune checkpoints. Immuno-oncology therapies – using antibodies or recombinant forms of receptors or ligands – can disrupt these molecular checkpoints stimulating an anti-tumour response and potentially conferring long-term cancer immunity. As well as ultimately benefiting patients, the identification of novel immune checkpoint interactions presents a significant commercial advantage to drug discovery companies by opening up new targets for therapeutic development.

AIM

Despite the recent surge in interest in this area, the molecular mechanisms for several key immune checkpoint interactions have yet to be elucidated due in large part to the limitations of standard techniques. Plasma membrane protein array (PMPA) screening has been successful in identifying key receptors for both orphan ligands[1]and for phenotypic molecules discovered using functional studies[2].

Here we demonstrate the power of the PMPA approach in uncovering immune checkpoint interactions using the ligands CTLA4 and CD27.

METHODS

Both CTLA4-hFc and CD27-hFc were screened for binding with approx 4,500 human plasma membrane proteins that were individually over-expressed in HEK293 cells as outlined below.

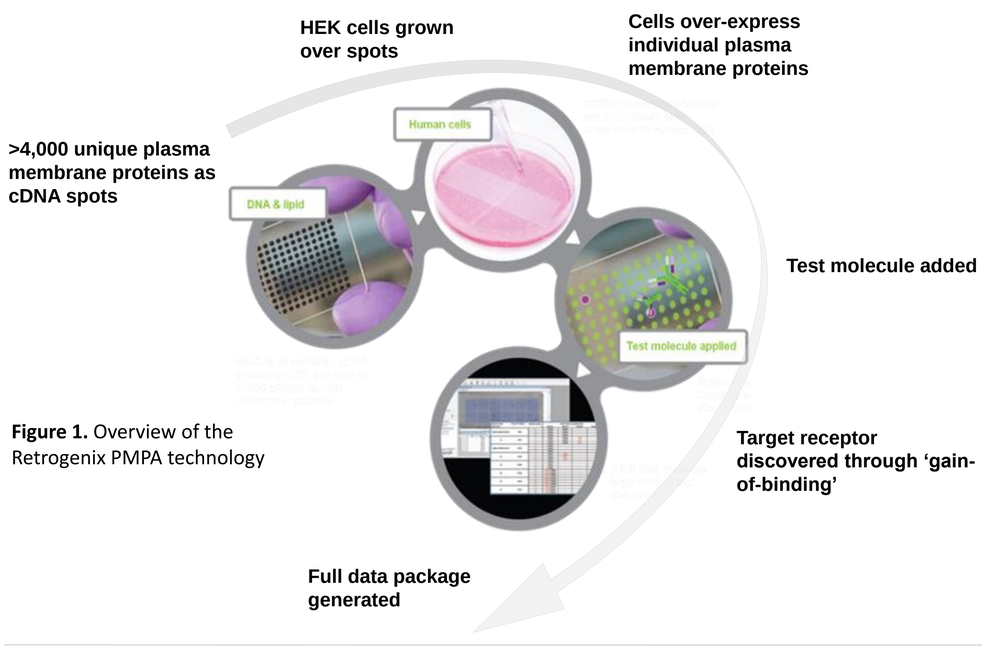


Figure 1. Overview of the Retrogenix PMPA technology

Primary screens: 13 slides were spotted in duplicate with expression vectors encoding plasma membrane proteins. Human HEK293 cells were overlaid and reverse-transfected. Each Fc-tagged test ligand was added to all slides and gain of binding detected using an Alexafluor647 anti-hIgGFc secondary antibody.

Confirmation screen: Positive 'hits' were confirmed and tested for specificity.

RESULTS

CTLA4: Both isoforms of the CD86 receptor were successfully identified along with the CD80 receptor. No secondary targets or false positive results were reported using PMPA.

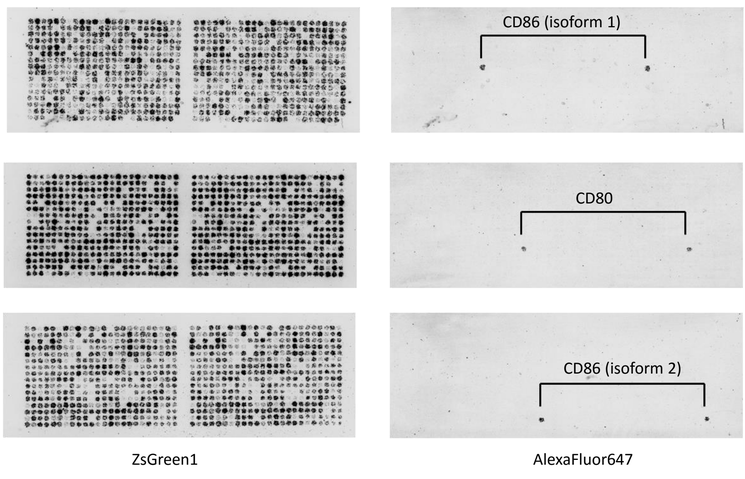


Figure 2. Results from primary screen for CTLA4 showing 3 of 13 slides spotted in duplicate. ZsGreen1 acts as transfection control and provides spot co-ordinates to identify the primary hits: CD86 and CD80.

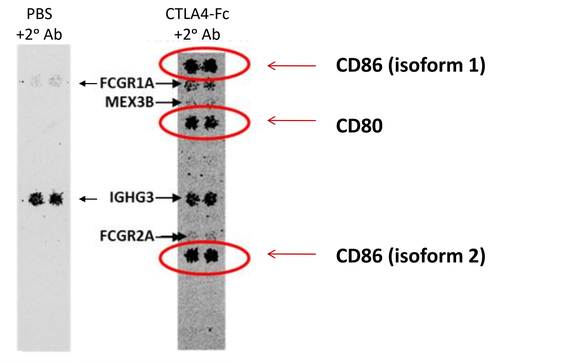


Figure 3. Confirmation screen for hits that were specific for CTLA4-Fc.

CD27: The PMPA screen correctly identified the known receptor, CD70, along with two further specific, weak intensity hits. These data point to an additional two receptors for CD27 which warrant further investigation.

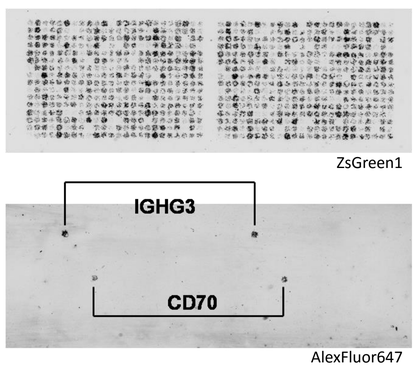
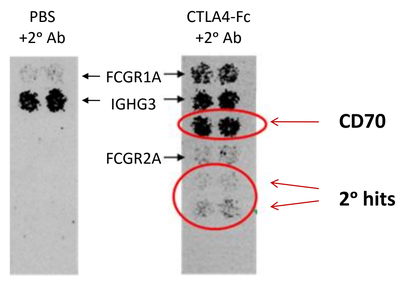


Figure 4. Primary screen for CD27 showing 1 slide (of 13) spotted in duplicate. ZsGreen1 acts as transfection control and provides spot co-ordinates to identify specific hits, CD70 and IGHG3.

Figure 5: Confirmation screen for hits that were specific for CD27-Fc.



Immune checkpoint protein (ECD-hFc)	Known counter receptor(s) identified?	False +ves?	Comments
CTLA4	Yes – CD80 &CD86	No	Both isoforms of CD86 hit
CD27	Yes – CD70		Two additional receptor hits – weak intensity, but specific. Need validating

Table 1. Summary of Retrogenix PMPA results

IMPACT

These results demonstrate an efficient method for rapidly identifying specific ligand-receptor interactions in human cells. The technology is routinely used to uncover novel targets in the development of immuno-oncology therapies.

References:
1. Turner L. *et al.* (2013). *Severe malaria is associated with parasite binding to endothelial protein C receptor.* **Nature** 498:502–505.
2. Sandercock AM *et al.* (2015) *Identification of anti-tumour biologics using primary tumour models, 3-D phenotypic screening and image-based multi-parametric profiling.* **Molecular Cancer** 14:147