High-density peptide microarrays for HLA-Class I immunopeptidome profiling in cancer

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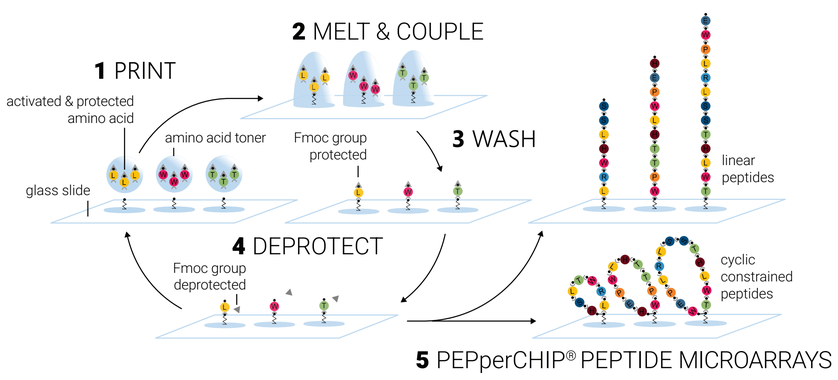
KEY FINDINGS

In this study, we show the accuracy of PEPperPRINT microarrays in identifying immunogenic neoepitopes of human melanoma samples. Our innovative platform offers rapid identification of neoepitopes based on their binding to different recombinant human HLA molecules, presenting several advantages over other *in silico* and *in vitro* methodologies:

- The peptide library representing the patient-specific neoantigens undergoes screening with recombinant human HLAs, obviating the necessity for patient samples or scarce biological material
- Discovery occurs within a cell-free system, mitigating the potential interference from cells, pathogens or other protein-based culture material
- The data provided doesn't rely on predictions that would limit the number of analyzed neoantigens or involve complex computational methodologies

PEPperCHIP® Peptide Microarray Platform Technology

High-density PEPperCHIP® Peptide Microarrays are generated by digital laser printing on standard glass slides using a proprietary laser printer with 24 individual amino acid toners. For array production, amino acid toners are simultaneously printed with high precision on their respective positions on the glass slides.



Benefits

- high spot density/high content (1,200 peptides/cm^2)
- digital printing flexibility
- high peptide quality with routine double couplings
- very low material consumption

Figure 1. The PEPperCHIP® Peptide Microarray printing process.

Background

The latest development regarding cancer immunotherapy is a personalized treatment that demonstrates enhanced specificity and sensitivity. At the level of T-cell immunotherapy, this personalization is taken a step further, where cancer vaccines and adoptive T-cell therapies (ACT) are developed based on the tumor antigen and/or T-cell repertoire identified in individual tumors.

Adoptive cell therapy involves the isolation of a patient's T-cells and reinfusing them back after a series of modifications that activate T-cells to recognize tumor-specific antigen derived peptides, also known as neoepitopes. Neoepitope identification is very challenging due to the high number of polymorphisms in human leukocyte antigen (HLA) molecules and presented peptides.

Although next-generation sequencing unveils neoepitope candidates by identifying tumor-specific somatic mutations at the transcriptome level, it can not provide information for the peptides presented by HLA molecules. Each HLA molecule has a specific peptide preference for binding and the set of peptides presented in HLA molecules is referred to as immunopeptidome or HLA ligandome (Fig. 2).

Despite numerous efforts in the past decade to map antigen-specific T-cell repertoires, several challenges have constrained their success. These challenges include limitations in instrument sensitivity, labor-intensive processes, and the requirement for substantial amounts of patient material, in addition to dependence on predictions and complex computational methodologies.

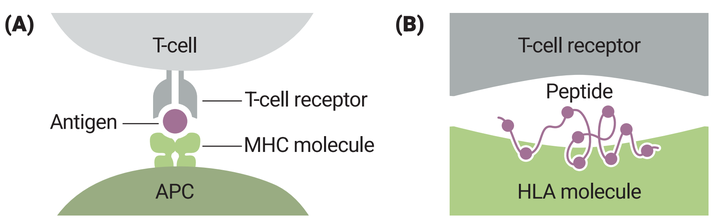


Figure 2. Antigen-specific T-cell activation. (A) The activation of T-cells is antigen-specific and requires recognition of peptides that are bound to HLA molecules (peptide-HLA) by the T-cell receptor. (B) HLA genes have the highest degree of polymorphism in human genome and resulting HLA proteins have various peptide binding motifs.

Objective and Significance

Here, we present a novel chip-based methodology that effectively surmounts current limitations in the field, allowing for the discovery of epitope-specific T-cell responses in a remarkably brief timeframe of just five weeks. The primary objective of this study was to explore tumor-associated peptides that could potentially be presented to T-cells of melanoma patients carrying the HLA-A2 allele. Given the pace of disease progression, our method could significantly contribute to advancing personalized immunotherapies for cancer, serving as a life-saving strategy.

Methods

In this study, we employed high-density peptide microarrays, a robust and efficient method capable of simultaneously screening thousands of peptides against specific binders in a high-throughput manner. Amino acid sequences of 21 melanoma antigens were printed in an overlapping peptide library format, addition to the peptides displaying tumor-specific somatic mutations. To ensure comprehensive coverage, literature-derived and experimentally-confirmed HLA-A2-specific melanoma epitopes were incorporated into the same array as positive controls. The resulting peptide microarray contained 4125 peptides printed in duplicate, framed by HA (YPYDVPDYAG) quality control peptides, and screened for recombinant HLA-A2 protein binding. Data was analyzed by PepSlide® Analyzer software and the immunogenicity of the identified T-cell epitopes was validated through an ELISpot assay.

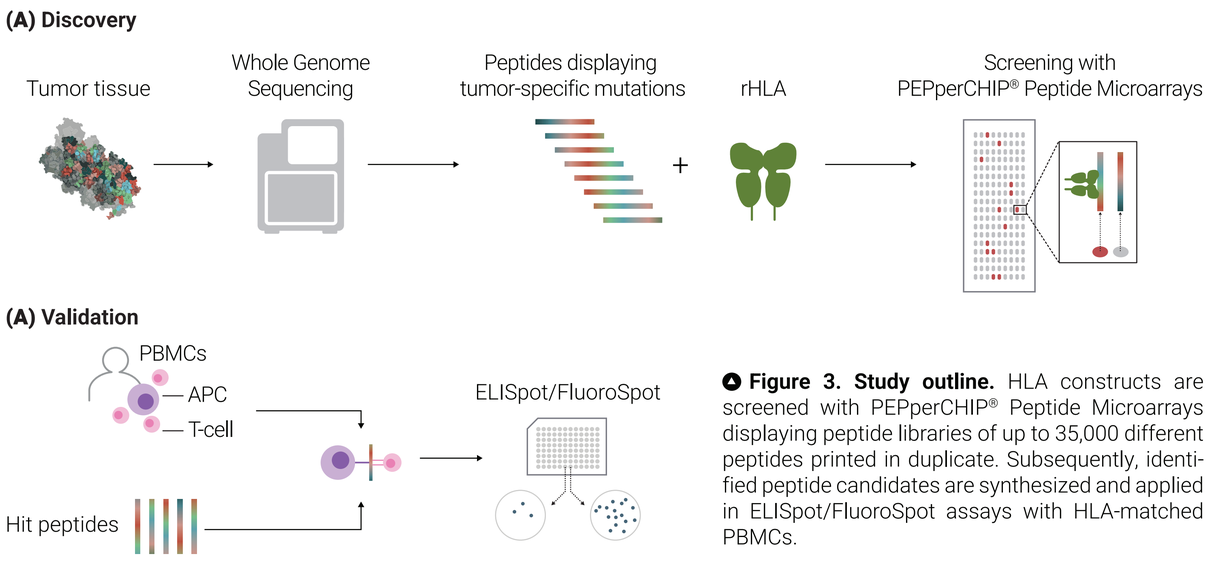


Figure 3. Study outline. HLA constructs are screened with PEPperCHIP® Peptide Microarrays displaying peptide libraries of up to 35,000 different peptides printed in duplicate. Subsequently, identified peptide candidates are synthesized and applied in ELISpot/FluoroSpot assays with HLA-matched PBMCs.

Results and Conclusions

Incubating the melanoma antigen microarray with recombinant human HLA-A2 construct resulted in the validation of 95 out of 100 positive control peptides, alongside new epitope discoveries. This approach can be easily transferred to all other immunogenicity screening of custom antigens against specific HLA constructs. In conclusion, our study shows the potential of PEPperCHIP® Peptide Microarrays in advancing our understanding of peptide-HLA interactions.

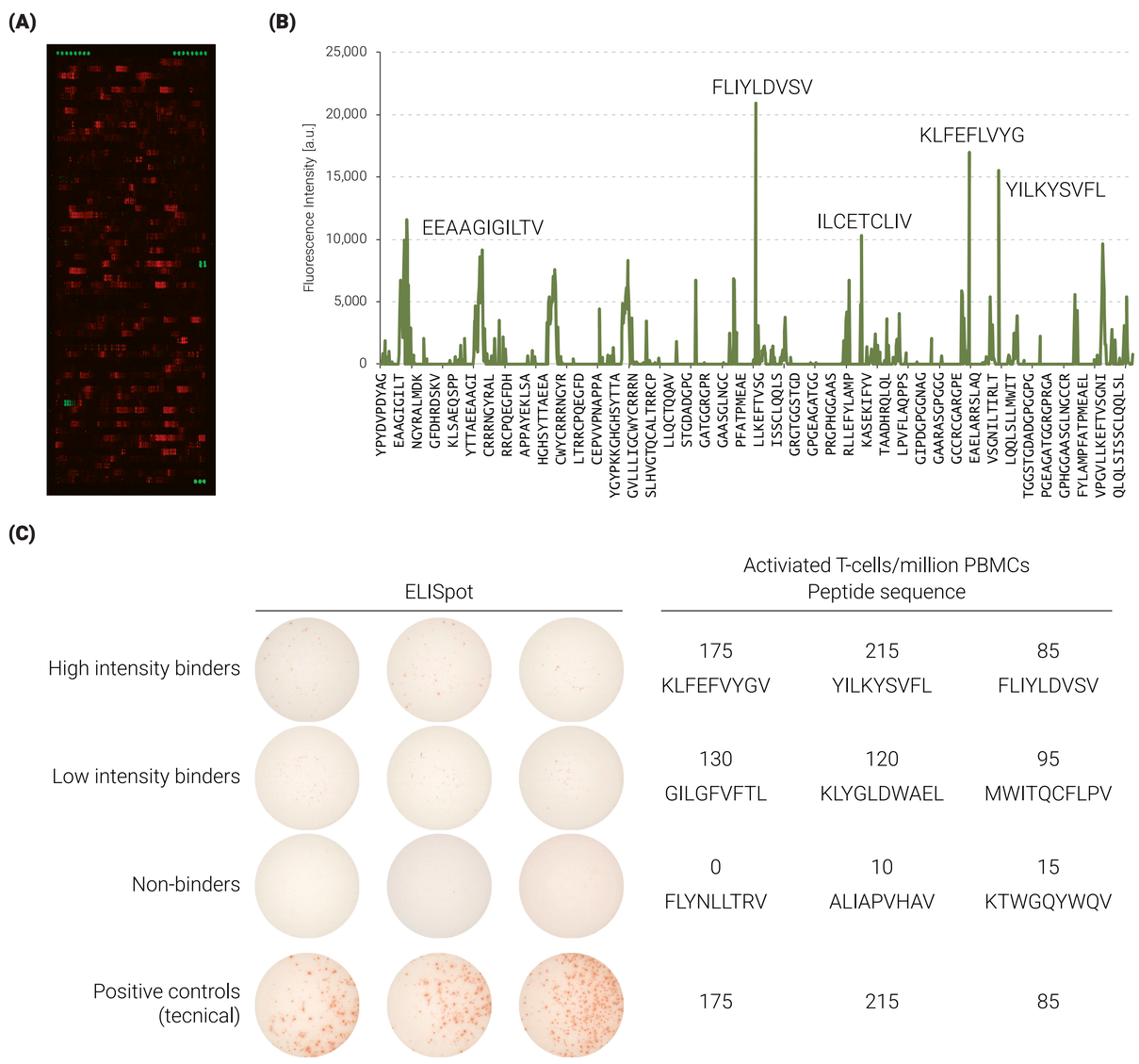


Figure 4. Discovery of T-cell receptor epitopes. (A-B) Example chip and intensity plot of the assay showing literature-derived positive controls in addition to the new discoveries. (C) For validation, 200,000 PBMCs per well were stimulated in a 96-well IFN-γ ELISpot plate for 24 hours with identified peptides. ELISpot was analyzed on an ImmunoSpot® Analyzer. Spots per 1 million PBMCs calculated.

Interested in working with our PEPperCHIP® Peptide Microarrays?
Visit our website to learn more and explore our related peptide microarrays and services for cancer research, or get in touch with Dr. Ebru Aydin Kurtulmus at **ebru.aydin.kurtulmus@pepperprint.com**.

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