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SYSTEMS AND METHODS FOR IDENTIFICATION OF MHC-I PEPTIDE EPITOPES USING MULTIPLEXED PEPTIDE RECEPTIVE MHC-I/CHAPERONE COMPLEXES

Tech ID: 33088 / UC Case 2019-975-0

BACKGROUND

The identification of high-affinity peptide epitopes displayed on MHC-I molecules is an important first step in understanding cell-mediated immune responses and in the development of targeted immunotherapies to treat infections or cancer. This task is typically addressed through the use

of highly sensitive mass-spectroscopy approaches and machine learning algorithms. However, this approach is hampered by peptide loss during the upstream purification step. The approach is also hampered by a lack of specificity in purification.

This technology involves the use of peptide-receptive MHC-I molecules in complex made using the TAPBPR chaperone. The peptide receptive MHC-I can be immobilized on chromatography columns or magnetic beads. They can provide unprecedented levels of highly specific peptide recovery

TECHNOLOGY DESCRIPTION

Proteins of the class I Major Histocompatibility Complex (MHC-I) play a pivotal role in orchestrating an adaptive immune response by alerting the immune system to the presence of developing infections and tumors in the body. Immune surveillance is achieved through the display of short (8-11 residue long) peptides derived from viral proteins (or mutated oncogenes) via a tight interaction with the MHC-I peptide-binding groove. Such peptide/MHC-I

protein complexes are assembled inside the cell and displayed on the surface of all antigen presenting cells where they can interact, primarily with T cell receptors

The MHC-I proteins are extremely polymorphic (more than 13,000 different alleles have been identified in the human population to date), and each allele can display an estimated 1,000-10,000 different peptides. This makes the prediction of actively presented MHC-I peptides challenging. The current state-of-the art for the identification of MHC-I displayed peptides is the use of Liquid Chromatography-Tandem Mass spectroscopy (LC/MS/MS). This technique allows the identification of the masses of thousands of peptides extracted from MHC molecules in relevant biological samples. The derived peptide sequences can be cross-referenced with protein sequence databases.

Although LC/MS/MS is an extremely powerful technique, the recovery of peptides from purified MHC molecules prior to LC/MS/MS using standard acid elution protocols is subject to losses during the peptide purification process. Up to 80% of relevant peptides are not recovered in most applications. This is a major challenge, as the lost peptides can be important targets for both understanding antigen processing and presentation processes and the development of immunotherapies to combat bacterial and viral infections and cancer.

MHC-I peptide libraries for peptidome analysis have historically been generated by acid extraction of the peptides from cells or by MHC-I immunoprecipitation and acid extraction. In addition to the 80% loss mentioned above, an unacceptably high percentage of peptides derived using the acid extraction from cell lysate or cell surface is nonspecific for the MHC-I. The comparison of peptide repertoires extracted from MHC-I-expressing and β 2-microglobulin knockout cells revealed that approximately 50% of the peptides identified as MHC-I peptide ligands are false positives not from an MHC class I binding groove.

CONTACT

Jeff M. Jackson
jjackso6@ucsc.edu
tel: .



INVENTORS

- ▶ Morozov, Giora
- ▶ Sgourakis, Nikolaos

OTHER INFORMATION

KEYWORDS

MHC-I epitope, T cell receptor specificity, T cell antigen recognition, CD8 T cell epitope, MHC-I peptide library

CATEGORIZED AS

- ▶ **Biotechnology**
 - ▶ Proteomics
- ▶ **Research Tools**
 - ▶ Reagents
 - ▶ Screening Assays

RELATED CASES

2019-975-0, 2018-408-0, 2020-251-0, 2020-284-0, 2020-297-0

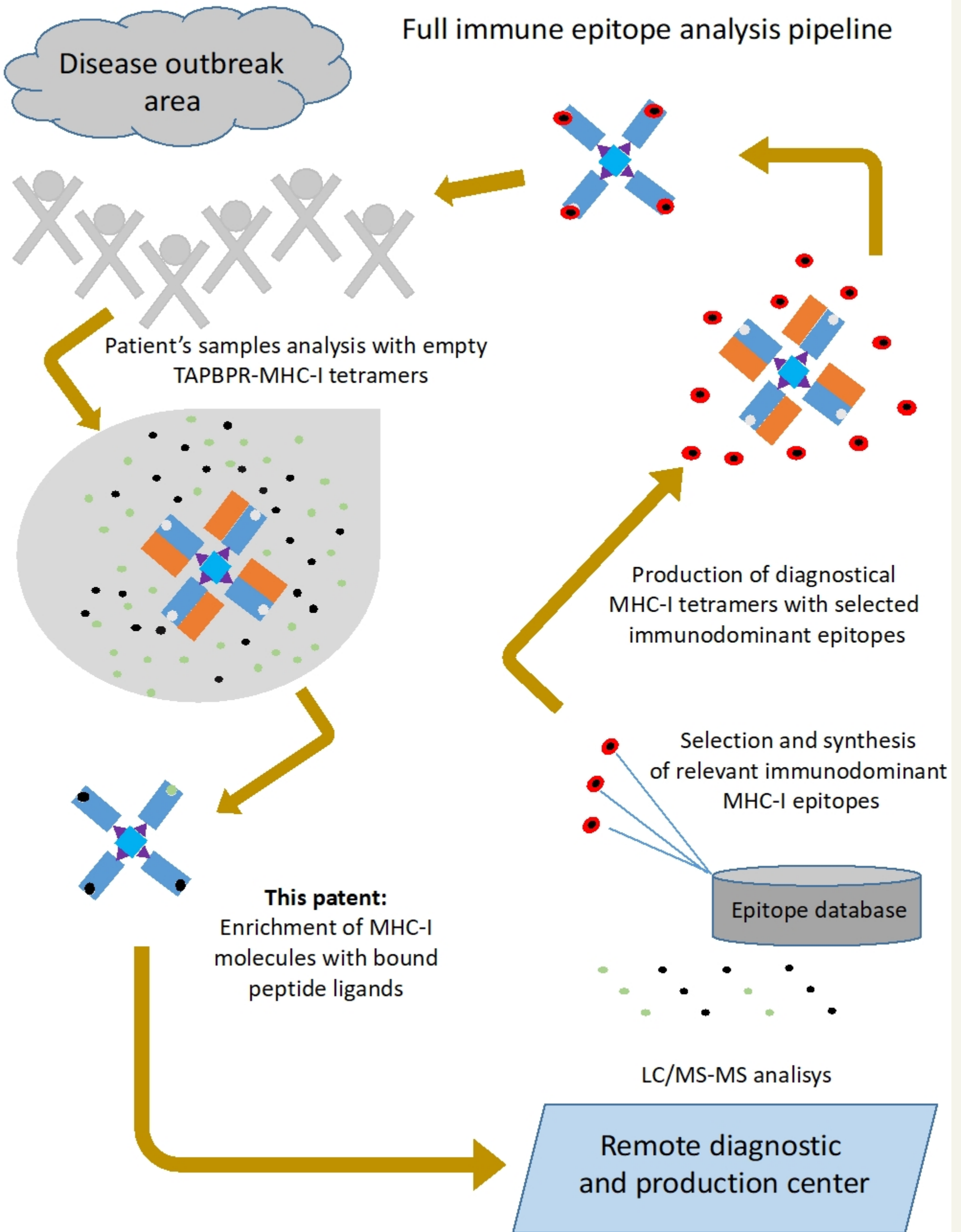
In addition, cells can express multiple (up to six) different MHC-I alleles, further complicating analysis. And such cells need to express high levels of MHC-I - which tumor and infected cells downregulate.

The use of highly purified, recombinant peptide receptive MHC-I molecules made using TAPBPR efficiently overcomes these bottlenecks. It provides the high concentration of MHC-I molecules necessary for reliable and sensitive peptidome analysis in samples of any origin.

The use of affinity tags attached to the recombinant proteins provides the specific binding for MHC-I molecules and diminishes contamination with non-specific peptides.

The method is modular and flexible enough to support a towards a range of applications, including performing immune repertoire characterization of patient samples. Examples are below:

Full immune epitope analysis pipeline



In this example, patient samples are collected at field sites and applied to columns containing peptide-receptive MHC-I made using TAPBPR. MHC-I molecules enriched with bound ligands can be purified and sent to centralized clinical/research centers for MHC-I epitope identification (e.g. using LC/MS/MS), cross referencing with epitope databases, or using machine learning techniques to predict specificities of enriched peptide ligands for various MHC-I alleles.

These peptides can then be used to produce diagnostic MHC-I tetramers for the immunodominant epitopes that can be sent back to the field for diagnostic and longitudinal surveys as well as testing the efficiency of vaccine or drug trials. The epitope data can also be used in vaccine development.

APPLICATIONS

Identification of MHC-I antigenic peptides

Generation of MHC-I peptide libraries from infected patients

Generation of MHC-I peptide libraries from cancer patients

Generation of MHC-I peptide libraries from autoimmune patients

Identification of Immunodominant Epitopes

ADVANTAGES

Minimal loss of antigenic peptide

Ease of use

Minimal false positives

INTELLECTUAL PROPERTY INFORMATION

Country	Type	Number	Dated	Case
United States Of America	Published Application	20230059548	02/23/2023	2019-975
European Patent Office	Published Application	4085069	11/09/2022	2019-975

RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- ▶ [Systems And Methods For Performing Peptide Exchange Reactions Using Placeholder Peptides And Catalytic Amounts Of The Molecular Chaperone TAPBPR](#)
- ▶ [Systems And Methods For Generating Peptide Deficient Hla-A*68:02 And Hla-A*24:02 Molecules](#)