A computational approach to predict off-target peptide selectivity of pMHC targeting therapeutics

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ABSTRACT

Major Histocompatibility Complex (MHC) molecules bind peptides derived from intracellular proteins forming peptide-MHC (pMHC) complexes which are recognized by the effector T-cells. The therapeutic molecules targeting pMHCs might cause off-target effects due to the similarity of the target peptide antigen to other pMHCs present on the surface of normal vital tissues. We present a computational approach for predicting off-target effects of pMHC targeting therapeutics such as T cells engineered to express a tumor-targeting chimeric antigen receptor (CAR) or a T cell receptor (TCR). We tested 39 peptides identified using this approach that could potentially result in off-target toxicity for 9 CARs and a TCR targeting HPV E6 epitope peptide (TIDHILECV) in complex with HLA-A*02:01. The CARs and the TCR showed minimum cross-reactivity for two of the peptides tested in Jurkat/T2 peptide loading assay.

INTRODUCTION

The presentation of peptides by major histocompatibility complex (MHC) class I is critical for the T cell adaptive immune response. Inside the cell, antigens are processed to generate peptides which are loaded onto MHC molecules to form peptide-MHC (pMHC) complexes. The peptide antigen’s anchor residues buried in the peptide binding cleft enable binding to HLA molecules. The binding solvent exposed residues are involved in interaction with the TCR.

The selectivity funnel method uses peptide similarity, HLA anchor residue preference, biochemical property difference and gene expression in normal tissue as filters to identify peptides that could potentially cause off-target effects for pMHC targeting therapeutics.

Identity score

- An identical amino acid to the target peptide at each position gets a score of +1
- The higher the Identity score, the more likely is for the peptide to bind the target

Anchor score

- Anchor positions and residue preferences for each target MHC class I allele were extracted from the IEDB database?
- The residue preferences at each anchor position were identified using the SMIM (stabilized matrix method).3,6
- The cutoffs used by Wang et al.5 to assess the impact of a position in binding were modified for each target MHC class I allele as illustrated below for HLA-A*01:01/HLA-A*02:01 and 9-mer peptides.
- Preferred residues: score <1 by SMIM
- Tolerated residues: 0<=score<1 by SMIM
- Penalized residues: score >=2 by SMIM

Incompatibility score

- Rationale: Amino acids with the similar biochemical properties match the same target
- Incompatibility score:
  - Amino acids are grouped based on their biochemical properties
  - A change in an amino acid in a different group is scored as +1
  - The higher the incompatibility score, the less likely is to be the peptide to bind the target

Cutoff setting

HLA-A*01: Cutoffs set based on the HLA peptide (see below for rationale)

- T-cells expressing the TCR against HLA-A*01-restricted MAGE-A3 peptide EVYDPSKYL resulted in fatal cardiac toxicity in two patients.7
- The presentation of HLA-A*01 peptide derived from the muscle protein Titin (ESPDHAYQY) on cardiac tissues in vivo was proposed to be the most likely cause of the off-target toxicity.8
- Titin peptide is used in determining the cutoffs for the Selectivity funnel.

Protocol test

HLA-A*02: Protocol test using EPSL2 peptide

- T cells expressing the TCR against HLA-A*02-restricted MAGE-A3/4 peptide QAIELYVFL cross-reacted with non-MAGE-A peptide from gene EPSL2 pulsed onto T2 cells.9
- EPSL2 peptide is used in testing the Selectivity funnel cutoffs set above.

SUMMARY

- A peptide selectivity prediction algorithm has been developed to identify peptides that could potentially cause off-target effects for pMHC targeting therapeutics.
- The selectivity funnel method uses peptide similarity, HLA anchor residue preference, biochemical property difference and gene expression in normal tissue as filters.
- The algorithm has been optimized using peptides from the literature that were proposed to present off-target toxicity.
- This approach has been successfully applied prospectively to test the selectivity of HPV E6 CARs and the TCR molecule against 39 peptides similar to the HPV E6 peptide (TIDHILECV).
- CARs and the TCR show minimum cross-reactivity to 2 out of 39 peptides tested (7-18 fold difference in activity)

REFERENCES