Lymphoma neoantigens

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Take Home Messages

- MHC binding prediction software can be quite accurate, but may not describe bona fide peptide antigens specific to lymphomas and other cancers.
- Empirical measurements with mass spectrometry can reveal a broad range of cell-specific antigens, including immunoglobulin idiotype lymphoma neoantigens.

Introduction

Our immune systems strike a balance between the vigilance required to kill cells compromised by infection or cancer with the tolerance needed to avoid harming normal cells. The dynamic and complex repertoire of antigenic peptides presented on major histocompatibility class I and class II proteins (MHC-I, -II) are the foundation of T-cell-driven adaptive immune responses. Immunotherapies targeting cancer-specific antigens (“neoantigens”) presented by MHC have high potential for improving rates of long-term, disease-free survival. However, neoantigen-targeting vaccines or engineered T-cells may be limited if bona fide targets are not known. Computational strategies for predicting MHC-binding peptides, when paired with subsequent binding and T cell activation assays, have revealed several cancer-specific antigens, particularly for melanoma and other high-mutation burden tumors. Applying this approach to low-mutation burden tumors, such as lymphomas, has not found similar success. Empirical antigen discovery directly from tumors could identify new classes of therapeutic targets which escape traditional prediction methods based on genomic sequencing data. Ultimately however, the field would benefit from improved approaches to rapidly predict and screen candidate antigens for their ability to stimulate T-cells in vivo.

Current state-of-the-art

MHC genes are the most variable regions in the human genome, with over 9000 MHC class I alleles and 3000 MHC class II alleles described to date. MHC-I tend to present 9-to-11-mer peptide sequences at their plasma membranes, but different allelic forms can vary widely in the range of sequences they can bind. This can complicate efforts to achieve universal immunotherapy reagents: even though two patients’ tumors may share a stereotypical mutation, potential MHC peptide ligands spanning this mutation might bind strongly to one patient’s MHC-I alleles and not bind at all to the other patient’s. Powerful computational tools such as netMHC have evolved over the past 15 years to model these varied peptide binding affinities such that robust predictions for common MHC class I alleles are now fairly routine. The greater diversity of peptides capable of binding MHC class II have posed a greater challenge for prediction tool development, but they are constantly improving. Armed with these tools, several research groups have taken advantage of the increasing availability of genome-wide exome sequencing data from tumors, and consequent tumor-specific mutation profiles. These studies have suggested an intuitive path towards personalized and targeted immunotherapy in melanoma: once cancer-specific mutations are identified by high throughput exome sequencing, mutation-bearing antigens can be predicted based on each patient’s expected MHC binding profiles. These can then be synthesized, and screened for T cell recognition or used as peptide- or RNA-based vaccines. A complementary and direct approach for MHC ligand identification was first described over 25 years ago in which MHC-peptide complexes are immunoprecipitated, peptide ligands are released, and subsequently identified by mass spectrometry. Where these initial studies identified just a handful of such antigens from cell lines, sample handling, mass spectrometry instrumentation and computational tools have advanced to the point where thousands of MHC-I and MHC-II ligands can be identified and quantified from milligram-quantities of primary tissue. Ongoing studies are revealing how MHC peptide repertoire reports cell state information which is orthogonal to more conventional transcription or proteome assays. Importantly, these empirical observations suggest that peptide ligands with predicted low binding affinity are frequently presented although they would not be prioritized by prediction tools. Thus, while mass spectrometry data can be used to restrict MHC binding predictions to those neoantigen peptides which are empirically presented, they can also reveal a wider range of antigens that have escaped prior notice. MHC binding and presentation alone, however, are insufficient predictors of a patient’s endogenous T-cell activities towards a putative neoantigen. Direct T-cell activity measurements, such as MHC tetramer staining, ELISpot and T-cell receptor sequencing are still required for linking specific mutations with adaptive immune responses (Figure 1).
In contrast to successful melanoma antigen prediction studies, our team initially attempted to predict over 100 neoantigen-related peptides for testing in mantle cell lymphoma patients, and found no measurable T-cell responses to them. We then subjected these and other patients’ tumors to our mass spectrometry-driven MHC-I and MHC-II peptide ligand discovery approach and found over 35,000 unique peptide ligands. None of these could be attributed to non-synonymous somatic mutations. However, noticing that immunoglobulin-derived peptides were highly represented in both MHC-I and MHC-II ligandomes, we characterized each tumors’ unique variable chain (idiotype) sequences, and searched our mass spectrometry data accordingly. We found that tumor-specific idiotype “neoantigens” were frequently presented by MHC class II, and surprisingly, were not presented by MHC class I. We then demonstrated that CD4 cells were capable of recognizing these antigens, and in one case, directly induce tumor cell killing in vitro. This study suggests that neoantigen identification efforts focused on non-synonymous somatic mutations may not be effective for lymphomas and other low-mutation burden tumors. However, specific knowledge of these cells’ biology – such as immunoglobulin variable chain recombination and hypermutation – led us to an alternate type of cancer neoantigen. Our study further shows that unusual T-cell subpopulations – for example granzyme B-expressing CD4+ T-cells – may be able to isolated and used for therapeutic purposes.

**Future perspectives**

Our observation that MHC-presented antigens derived from immunoglobulin variable chain sequences suggests that these lineage-specific rearrangements could be useful therapeutic targets in a broader array of B- and T-cell malignancies. Our data also suggest that MHC presents a wider array of antigens that are restricted to a particular cell type. Since many of these antigens are derived from intracellular proteins, they open the possibility for developing new assays, enrichment tools, and vaccination strategies which target different cell populations based on their presented peptides in a fashion that complements conventional cell surface marker approaches. Despite these advances, mass spectrometry-based MHC ligand measurement still requires millions to hundreds of millions of cells per assay. Ongoing improvements in sample handling and instrumentation stand to make this approach compatible with a wider range of clinical applications.

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**Figure 1.** Logical intersections between predicted and empirical MHC peptide ligands. Software like netMHC can robustly predict whether putative neoantigen peptide sequences have the ability to bind a given major histocompatibility complex (MHC) allele (green circle), but these predictions generally do not account for gene expression and other factors which could affect whether these peptides are actually presented in vivo. Mass spectrometry-based surveys of MHC ligand repertoires provide empirical evidence of antigen presentation without a priori requirements that peptide ligands bind an MHC with high affinity, or even that the MHC allele’s affinity preference is known (blue circle). Although the high-affinity, frequently presented union between these two sets might be enriched for antigens with greater therapeutic potential, additional empirical measurements are needed to test whether these candidates actually stimulate T cells (grey circle). In addition to the “Goldilocks” union of all three categories, each of the seven regions in the resulting Venn diagram describes antigen sub-classes worthy of further inquiry to better understand the range of cancer-specific antigens which could hold diagnostic or therapeutic utility.
specimens. Last, recent intriguing data suggest that unusual biochemical events, such as proteasome-mediated splicing could be responsible for an underappreciated degree of MHC ligand diversity. Future work will need to rigorously test these and other observations, and evaluate the extent to which they carry immunological significance.

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