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Peptide Presentation Is the Key to Immunotherapeutical Success

Wiebke C. Abels, Alexander A. Celik,
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Additional information is available at the end of the chapter

Abstract

Positive and negative selection in the thymus relies on T-cell receptor recognition of peptides presented by HLA molecules and determines the repertoire of T cells. Immune competent T-lymphocytes target cells display nonself or pathogenic peptides in complex with their cognate HLA molecule. A peptide passes several selection processes before being presented in the peptide binding groove of an HLA molecule; here the sequence of the HLA molecule's heavy chain determines the mode of peptide recruitment. During inflammatory processes, the presentable peptide repertoire is obviously altered compared to the healthy state, while the peptide loading pathway undergoes modifications as well. The presented peptides dictate the fate of the HLA expressing cell through their (1) sequence, (2) topology, (3) origin (self/nonself). Therefore, the knowledge about peptide competition and presentation in the context of alloreactivity, infection or pathogenic invasion is of enormous significance. Since in adoptive cellular therapies transferred cells should exclusively target peptide-HLA complexes they are primed for, one of the most crucial questions remains at what stage of viral infection viral peptides are presented preferentially over self-peptides. The systematic analyzation of peptide profiles under healthy or pathogenic conditions is the key to immunological success in terms of personalized therapeutics.

Keywords: HLA, peptides, peptide prediction, adoptive T-cell therapies, peptide-vaccination

1. Introduction

The immune system of all species has to be able to discriminate self and foreign (nonself) antigens to combat infections without eliciting autoimmune diseases. The presentation of self and nonself occurs through displaying cellular proteins on the cell surface by proteins of the major histocompatibility complex (MHC) gene cluster. In humans, the MHC locus is termed human leukocyte antigen (HLA) and comprises several gene loci with numerous different alleles for most of the genes [1]. One part of the genes is subsumed as HLA class I (HLA-I) with the gene products being expressed on virtually every nucleated cell in the human body. HLA-I molecules present peptides of intracellular proteins on the cell surface. Cytotoxic T cells (CD8+ T cells) as part of the adaptive immune system can recognize these peptide HLA-I (pHLA-I) complexes by the T-cell receptor (TCR) and scan simultaneously the HLA molecule and the peptide [2, 3] to discriminate between healthy and unhealthy cells, for example, virally infected cells. At the same time, natural killer (NK) cells that are part of the innate immune system scan the cell surface of HLA as well. These cells become activated when HLA-I is missing on the cell surface, for example, on virally infected or tumor cells [4].

Peptide loading on HLA-I is a complex mechanism and determines in addition to the HLA allele which peptides will be presented. The central part of this process is the peptide loading complex that is localized in the endoplasmic reticulum (ER). The HLA-I molecule, consisting of a heavy chain and a microglobulin, is stabilized by several chaperons since the structure is unstable when no peptide is bound. The transporter associated with antigen processing (TAP) imports protein fragments that are degraded in the cytosol by the proteasome into the ER. Depending on the sequence, these peptides are trimmed in different ways in the ER [5, 6]. The bridge between TAP and HLA-I is the chaperon tapasin (TPN) that facilitates peptide binding in the peptide binding groove [7].

Because the HLA gene cluster ranks among the most polymorphic region in the human genome [1] and most of these polymorphisms are located in the peptide binding region (PBR) [8, 9], these polymorphisms result in an abundance of structurally different pHLA entities. In this chapter, we focus on the interplay between HLA alleles, bound peptides and the interaction with immune receptors. It is highlighted that even minor differences in the HLA sequence can impact on the bound ligand or the pHLA structure. Every single peptide changes the overall structure of the HLA molecule. Structural alterations that differ from self-pHLA structures will be recognized by the immune system. Therefore, the last parts of the chapter demonstrate the advantage of established immunopeptidomes for immunotherapies.

2. Peptide selection and presentation

The viability of the immune system is governed by interactions between effector cell receptors and their cognate antigenic ligands. Immune effector cells survey HLA-I molecules on the surface of antigen-presenting cells by indirectly scanning the proteomic content of every single cell. The fundamental role of CD8+ T cells, the elimination of pathogens, is elicited through HLA-I molecules complexed to a peptide of foreign (e.g., viral) origin.

Positive and negative selection of T cells in the thymus is a critical step for the development of a mature functional immune system. Immune cells that have not developed immune tolerance against specific pHLA-I complexes during thymus selection will recognize these antigens as foreign. The allele-specific and patient-specific peptides that are presented on the cell surface shape the individual immune response. Even a single alteration in the peptide sequence can be recognized by immune effectors. Single alterations in the sequence of the HLA heavy chain might not affect which peptide sequences can be bound but could lead to a modified overall pHLA-I structure or might affect the strength of peptide binding resulting in pHLA-I complexes with different half-life times. Besides the influence of amino acid (AA) exchanges within the heavy chain, peptides might undergo competition in patients who carry alleles with the same peptide-binding motif. For those reasons, the presentation of a given peptide is dependent on the HLA type and the health status of the patient. Half-life times of pHLA
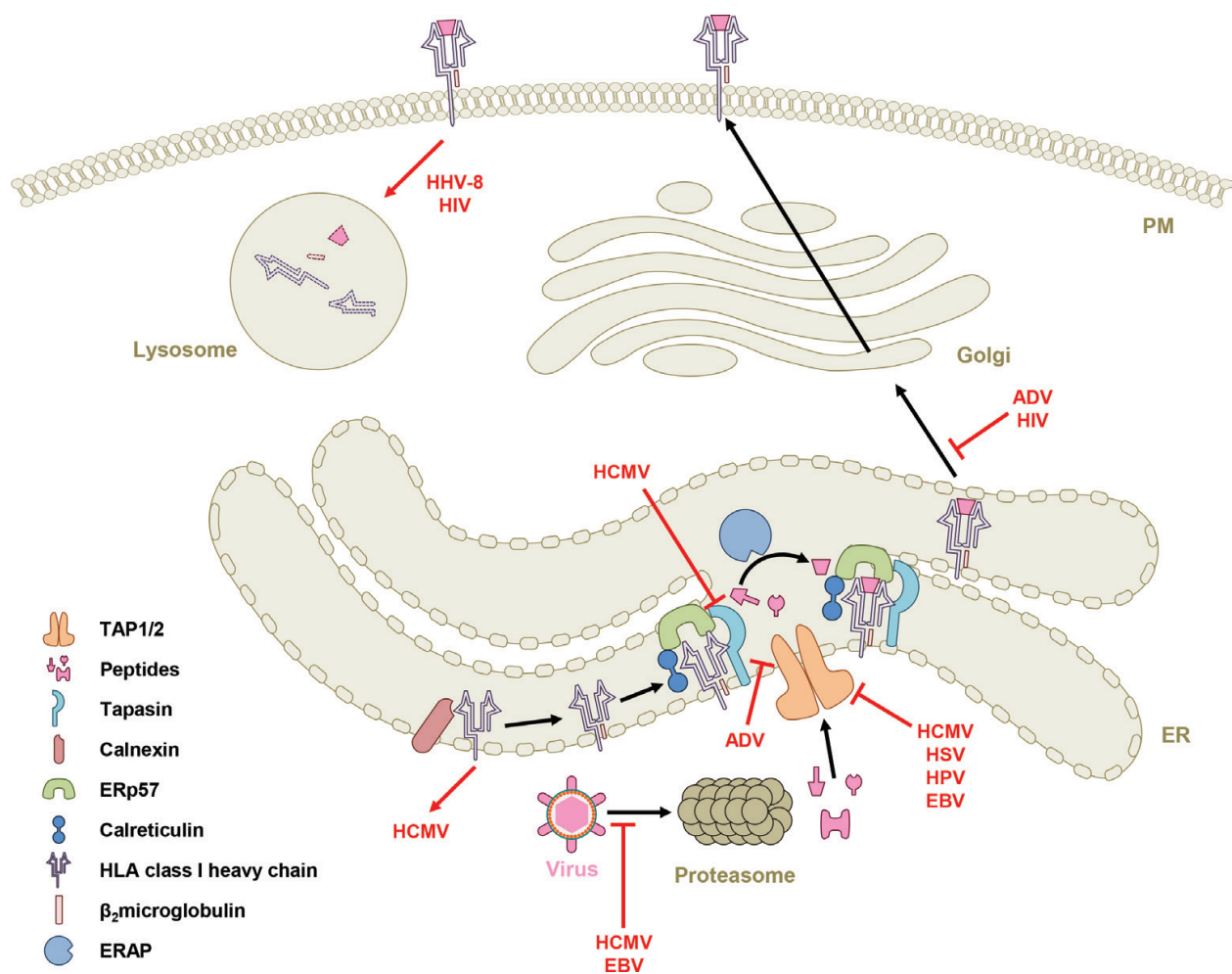

Figure 1. Viral interference with HLA class I peptide presentation. Depicted are targets for viral interference with peptide presentation on HLA-I molecules. HLA-I maturation and surface presentation of peptides are blocked through different mechanisms early postinfection. Most viruses directly target peptide loading through TAP and peptide optimization by tapasin (HCMV [104, 105], HSV [106], HPV [107], ADV [108], EBV [109]). Additionally, cell surface expression is impeded by retention of HLA-I in the ER (ADV [110], HIV [111], HCMV [112]) or rapid degradation of surface molecules (HHV-8 [113], HIV [114]). Other possibilities include dislocation of the HLA-I heavy chain before any peptide loading can occur (HCMV [115, 116]) and inhibition of proteasomal processing of viral proteins by the host cell (EBV, HCMV [117, 118]).

complexes influence the phenotype and/or functionality of T cells. That has to be taken into account when choosing pHLA-I targets for T-cell therapeutics. Based on these facts the importance of knowledge about allelic peptide specificity becomes obvious. Peptides have to pass several intracellular filters and processing steps before being presented to immune effectors cells. The bottleneck is the peptide loading complex (PLC). Not every available peptide in a cell would be necessarily bound to an HLA molecule and displayed at the cell surface since peptides undergo peptide competition during recruitment through the PLC. The PLC consists of several proteins; each has a specialized function for peptide selectivity and specificity. Proteins of this complex are dedicated targets for viral interference and thus viral immune evasion (**Figure 1**).

The HLA heavy chain has to adopt a peptide-receptive form and complex with certain proteins of the PLC. It could be demonstrated that certain allelic variants interact differently with proteins of the PLC and thus are more prone to present peptides of malignant origin. Those HLA subtypes can differ from alleles that are strictly dependent on the association with the PLC for peptide loading only by a single amino acid within the heavy chain, altering the structural interface that interacts with the PLC [10–12]. Especially the interaction of TPN, a protein that mediates the binding of high-affinity peptides into the HLA-I PBR, with the HLA-I molecule, is of exquisite importance to produce stable pHLA-I complexes that persist on the cell surface. However, few allelic HLA variants are able to present peptides without the assistance of TPN. That enables those alleles to continuously present viral peptides at an infectious stage where viral interference with TPN occurs; however, the presentation of self-peptides that did not take part in negative T-cell selection would be facilitated and might lead to uncontrollable autoimmune reactions. HLA-I variants that select and load peptides without the assistance of TPN are likely to present a broad range of low-affinity viral-derived peptides during an infection. However, the presentation of viral peptides during an active viral infection is a rare event since still self-peptides are present intracellularly and would compete with viral peptides to fit into the PBR. The viral peptides that would reach the cell surface complexed to an HLA-I molecule could hardly be predicted by peptide prediction tools.

3. Peptide specificity

Due to the fact that peptides undergo different selection steps before being presented by an individual HLA allele, it has to become clear that the individual HLA profile is the major and most distinguished obstacle. Every HLA allele differs from another by the composition of AAs in and/or outside the peptide-binding groove, resulting in allele-specific profiles of the bound peptides [13–15]. Therefore, the knowledge of an individual peptide binding profile can be used as precedence for the measurement of permissivity between HLA subtypes. The sequence, length and immunogenicity of a given peptide determine the half-life time of the whole pHLA complex and furthermore the specificity and reactivity of their cognate immune receptor.

Several studies demonstrated the impact of the sequence and feature of HLA-bound peptides on receptor recognition. This observation holds true for T-cell receptors of the adaptive immune system as well as for NK-cell receptors of the innate immune system. We recently could highlight the potential of the nonclassical HLA-I molecule HLA-E to select and present peptides of extraordinary length and their effect on differential NK-cell recognition. For the nonpolymorphic HLA-E molecule only two functional variants exist distinguished by a single AA difference. That would imply that HLA-E is, in regard to its peptide profile, invariant. However, by sequencing their bound ligands, we found both alleles presenting a different set of peptides [16]. Since HLA-E is an intermediate molecule for the adaptive and innate immune system, supporting the non-PLC-dependent presentation of peptides during HLA downregulation episodes, the invariability of this molecule would certainly make biological sense. However, the finding that HLA-E subtypes differ in their immunity was somehow unexpected. Reconstitution of empty HLA-E molecules with the designated peptides on the surface of artificial APCs resulted in a peptide-specific immune recognition [17].

Two types of NK-cell receptors interact with HLA-I molecules, killer cell immunoglobulin-like receptors (KIRs) and C-type lectin-like receptors. The former ones are highly polymorphic and polygenic in the population and recognize HLA-A, -B and -C alleles [18], whereas the latter ones bind among others to HLA-E [19, 20]. CD8+ T cells recognize endogenous HLA alleles and assess their immune status by virtue of the presented peptide. The display of different peptides thus allows for precise monitoring of the immune status of the cell through the adaptive immune system. However, this also means that these cells have to be primed on the recognition of specific peptides that are usually derived from endogenously expressed proteins [21]. The presented peptide repertoire can be altered by aberrant protein expression as well as the presence of foreign proteins (e.g., viral proteins) in the cell. To counter recognition by CD8+ T cells, many viruses have developed immune evasion strategies that specifically target HLA peptide loading and presentation. For instance, the HCMV protein US6 interferes with peptide translocation at the ER thus depriving the available peptide pool or even directly procures that the HLA heavy chain is degraded through US2 or US11 [22]. However, in the event of such disrupted HLA-I presentation, NK cells become activated. Although NK cells do not recognize the specific HLA allele, the absence of HLA expression on the cell surface triggers NK-cell activation. Nevertheless, NK cells can still recognize certain peptides in the context of the nonclassical HLA molecule HLA-E that presents a very narrow set of peptides derived from the signal sequence of other HLA class I molecules. These peptides are 9 AA in length and are anchored preferably by Met at peptide position p2 and Leu at pΩ, whereas positions p4, p5 and p6 are accessible to the solvent [23, 24]. On NK cells, HLA-E in combination with these peptides is recognized by inactivating the NKG2A/CD94 heterodimeric receptor complex [25]. In the absence of HLA class I molecules or during cell stress, HLA-E was shown to present noncanonical peptides of different length [16, 17], for example, the Hsp60-derived peptide QMRPVSRVL that causes loss of recognition by NKG2A/CD94 [26] or the HIV Gag-derived peptide AISPRTLNA that causes HLA-E upregulation [27]. In the case of HCMV, a peptide from the UL40 protein that closely resembles the sequence of leader

peptides from certain HLA-C allotypes is provided to stabilize HLA-E expression in infected cells. However, in individuals negative for these HLA-C allotypes, the UL40-peptide constitutes the presentation of nonself on HLA-E and can thus elicit a CD8+ T-cell response [19, 28]. Additionally, HLA-E in complex with other pathogen-derived peptides was shown to stimulate CD8+ T-cell responses. For instance, the Epstein–Barr virus-derived peptide SQAPLPCVL was shown to be recognized by the αβTCR of a CD8+-CD94/NKG2C+ T-cell clone [29, 30] described HLA-E-restricted *Salmonella enterica serovar Typhi*-specific CD3+CD8+CD4−CD56− T cells. These diverse interactions demonstrate the subtle interaction of innate and adaptive immunity through the presented peptide on HLA-E.

4. Peptide binding prediction, bioinformatic tools

To identify peptides that would be suitable for application in cellular therapeutic strategies, certain properties have to be analyzed: (1) the peptide-binding motif of the HLA allele of choice and (2) the HLA allele-specific features of the bound peptides such as length and topology. There are several bioinformatic tools that enable scientists and clinicians to predict peptides that would be presented by a certain HLA allele, yet, these tools do not consider allele-specific features and immune dominance of peptides. The kinetics of antigen expression and the competition of peptides to be preferentially bound and presented are also not considered by these bioinformatics prediction tools. Most data available in these tools are based on experimental peptide data (**Tables 1** and **2**). However, it remains unclear if those predicted peptides would ever be naturally presented. Peptides predicted from for example a viral protein would not necessarily be processed, selected and/or presented by the respective patient awaiting T-cell therapy. Therefore, the pathogen- or peptide-specific T cells that would be transplanted might not be able to find their mutual pHLA molecule. An example of the first successful adoptive transfer of virus-specific T cells described the transfer of HCMV-specific T cells and their reconstitution of antiviral immunity in an immune-deficient bone marrow transplant recipient [31]. The technique of adoptive T-cell transfer could be further improved leading to the selection of specific T-cells based on IFN-γ secretion or pHLA multimer staining and selection following antigen stimulation [32–34]. Both techniques bear the imperative to know which viral peptides are presented on the particular HLA subtype of, for example, HCMV-infected cells. So far, few HLA-restricted peptides have been studied. The majority of peptides are derived from the well-characterized phosphoprotein (pp)65 or the immediately early (IE)1 protein, however, not for every patient responses against these two proteins are immunodominant [35, 36]. Best studied are the pp65-derived peptides NLVPMVATV and TPRVTGGGAM, restricted to HLA-A*02:01 and HLA-B*07:02, respectively. Those peptides are described to induce extremely strong T-cell responses [37–42]. Yet, these peptides have been computationally predicted [43] but not been isolated from HLA molecules. Thus, it remains unproven if they would ever be naturally presented. That might be an explanation for the failure of long-term T-cell transfers [33, 44, 45].

Name	Application	Methods	Ref.	Number of HLA class I alleles	Number of HLA class II alleles	Peptide length	Other species	URL
BIMAS	Predicts half-time of dissociation of peptides from protein sequences	Coefficient tables	[58]	41 inc. supertypes	0	8–10	No	https://www-bimas.cit.nih.gov/molbio/hla_bind/
EpiJen	Predicts peptide binding from protein sequence (proteasome cleavage, TAP binding and MHC binding)	Multi-step algorithm	[59]	18	0	9	No	http://www.ddg-pharmfac.net/epijen/EpiJen/EpiJen.htm
hla_a2_smm	Predicts binding affinity of peptides, high affinity HLA-A2 binding peptides from protein sequence and mutated peptides with higher affinity	SMM pair coefficients	[60]	1	0	9–10	No	https://zlab.bu.edu/SMM/
IEDB T Cell Epitope Prediction Tools	Predicts T cell epitopes from proteins (MHC binding, processing and immunogenicity)	Several tools can be chosen	[61–64]	77	n/s	Class I:8–14 Class II: n/s	Chimpanzee, cow, gorilla, macaque, mouse, pig, rat for MHC class I; mouse for MCH class II	http://tools.iedb.org/main/tcell/
Mappp	Predicts antigenic peptides to be processed and presented by MHC class I from peptide or protein sequence	Uses BIMAS or SYFPEITHI for binding prediction	[65]	35 inc. supertypes	0	8–10	Mouse, cattle	http://www.mpiib-berlin.mpg.de/MAPPP/index.html http://www.mpiib-berlin.mpg.de/MAPPP/binding.html
MHC2MIL	Predicts binding affinity of MHC-II peptides from protein sequence	MIL	[66]	0	26	9–25	No	http://datamining-iip.fudan.edu.cn/service/MHC2MIL/index.html
MHC2PRED	Prediction of MHC class II binders	SVM	[67]	0	38 inc. supertypes	9	Mouse	http://crdd.osdd.net/raghava/mhc2pred/index.html

Name	Application	Methods	Ref.	Number of HLA class I alleles	Number of HLA class II alleles	Peptide length	Other species	URL
MHCBN	Database with information about allele specific MHC binding peptides, MHC nonbinding, TAP binding, TAP nonbinding peptides and T-cell epitopes	Database	[68, 69]	n/s	n/s	n/s		http://crdd.osdd.net/raghava/mhcbn/index.html
MHCMIR	Predicts binding affinity and levels of MHC-II peptides from peptide or protein sequence	MIR	[70]	0	13	All	Mouse	http://ailab.ist.psu.edu/mhcmir/predict.html
MHCPRED	Predicts binding affinity of peptides to MHC class I and II molecules and to TAP from protein sequence and calculates binding affinity for heteroclitic peptides	Additive method, partial least square regression	[71–73]	11	3	9	Mouse	http://www.ddg-pharmfac.net/mhcpred/MHCPred/ http://www.ddg-pharmfac.net/mhcpred/MHCPred/pepLib.html
MMBPred	Predicts mutated high affinity and promiscuous MHC class-I binding peptides from protein sequence, epitope enhancement, 1–3 AAs mutation of nonamer peptides	QM	[74]	40 inc. supertypes	0	9	Rhesus macaque, mouse	http://crdd.osdd.net/raghava/mmbpred/
MULTIPRED	Predicts binding of peptides to HLA class I and class II DR supertypes and individual genotypes	Uses NetMHCpan and NetMHCIIpan	[75]	13 supertypes	13 supertypes	8–11 for HLA class I and genotype 9 for HLA class II	No	http://cvc.dfci.harvard.edu/multipred2/index.php

Name	Application	Methods	Ref.	Number of HLA class I alleles	Number of HLA class II alleles	Peptide length	Other species	URL
NetCTL	Predicts CTL epitopes in protein sequences (Cleavage, TAP transport, HLA class I binding)	ANN	[76]	12 supertypes	0	9	No	http://www.cbs.dtu.dk/services/NetCTL/
NetMHC	Predicts binding of peptide to MHC class I molecules from peptide or protein sequence	ANN	[19, 77]	81 (or 12 supertypes)	0	8–14	Chimpanzee, rhesus macaque, mouse, cuttle, pig	http://www.cbs.dtu.dk/services/NetMHC/
NetMHCcons	Predicts binding of peptides to any known MHC class I molecule from peptide or protein sequence	Consensus (NetMHC, NetMHCpan and PickPocket)	[78]	User specified	0	8–15	Chimpanzee, gorilla, rhesus macaque, mouse, cuttle, pig	http://www.cbs.dtu.dk/services/NetMHCcons/
NetMHCII	Predicts binding of peptides to HLA-DR, HLA-DQ, HLA-DP from peptide or protein sequence	ANN	[79, 80]	0	26	variable	Mouse	http://www.cbs.dtu.dk/services/NetMHCII/
NetMHCIIpan	Predicts binding of peptides to HLA-DR, HLA-DQ, HLA-DP from peptide or protein sequence	ANN	[81, 82]	User specified	0		Mouse	http://www.cbs.dtu.dk/services/NetMHCIIpan/
NetMHCpan	Predicts binding of peptides to any known MHC class I molecule from peptide or protein sequence	ANN	[19, 83, 84]	User specified	0	8–14	Chimpanzee, gorilla, rhesus macaque, mouse, cuttle, pig	http://www.cbs.dtu.dk/services/NetMHCpan/
nHLAPred: ANNPred	Predicts MHC Class I binding regions in proteins	ANN	[85]	26 inc. supertypes	0		Mouse	http://crdd.osdd.net/raghava/nhlapred/neural.html
nHLAPred: ComPred	Predicts MHC Class I binding regions in proteins	ANN/QM	[85]	59 inc. supertypes	0		Rhesus macaque, mouse	http://crdd.osdd.net/raghava/nhlapred/comp.html

Name	Application	Methods	Ref.	Number of HLA class I alleles	Number of HLA class II alleles	Peptide length	Other species	URL
PREDPEP	Predicts binding of peptides to HLA class I from peptide or protein sequence	Published coefficient tables	[86]	6	0	8–10 (dependent on the allele)	Mouse	http://margalit.huji.ac.il/Teppred/mhc-bind/index.html
ProPred	Predicts MHC Class II binding regions in an antigen sequence	QM	[87]	51	0		No	http://crdd.osdd.net/raghava/propred/
ProPred I	Predicts MHC Class I binding regions in an antigen sequence	QM	[88]	39 inc. supertypes	0		Mouse, cattle	http://crdd.osdd.net/raghava/propred1/index.html
Rankpep	Predicts binding of peptides to MHC class I and class II molecules from peptide or protein sequence	PSSM	[89–91]	n/s	n/s	Dependent on the allele		http://imed.med.ucm.es/Tools/rankpep.html
svmhc	Predicts binding of peptides to MHC class I molecules from peptide or protein sequence	SVM, uses MHCPEP or SYFPEITHI	[92, 93]	31	0	8–10 (dependent on the allele)	No	http://svmhc.bioinfo.se/svmhc//
SYFPEITHI	Database of MHC ligands and peptide motifs and epitope prediction	Matrix/motif-based, published motifs	[94]	33	6	8–11 for HLA class I; 15 for HLA class II	No	http://www.syfpeithi.de/
TEPITOPEpan	Predicts tissue-specific binding of peptides to MHC class II molecules from peptide or protein sequence	PSSM	[95]	0	50	9–25	No	http://datamining-iip.fudan.edu.cn/service/TEPITOPEpan/index.html

Abbr. HMM = hidden Markov model, SVM = support vector machine, PSSM = position-specific scoring matrix, QM = quantitative matrices, ANN = artificial neuronal networks, SMM = stabilized matrix method, MIL = multiple instance learning, MIR = multiple instance regression, n/s = not specified.

Table 1. Listing of peptide prediction tools available on the web [Accessed November 2017].

Name	Underlying database/data source	URL for matrices/training data
BIMAS	Coefficient tables deduced from the published literature by Dr. Kenneth Parker, Children's Hospital Boston	https://www-bimas.cit.nih.gov/cgi-bin/molbio/hla_coefficient_viewing_page https://www-bimas.cit.nih.gov/molbio/hla_bind/hla_references.html
EpiJen	AntiJen [96, 97], SYFPEITHI [94]	http://www.ddg-pharmfac.net/antijen/AntiJen/antijenhomepage.htm
hla_a2_smm	BIMAS [58], SYFPEITHI [94], data described in Peters, Tong [60]	https://zlab.bu.edu/SMM/
IEDB T Cell Epitope Prediction Tools	IEDB [61], Sette lab, Buus lab, uses diverse predictions methods (see webpage)	http://tools.iedb.org/mhci/download/ http://tools.iedb.org/mhcii/download/
Mappp	BIMAS [58], SYFPEITHI [94], coefficient tables deduced from the literature by Kenneth Parker, Children's Hospital Boston	—
MHC2MIL	Data by Wang, Sidney [98]	—
MHC2PRED	JenPep [19], MHCBN [68]	—
MHCBN	MHCBN [68]	—
MHCMIR	IEDB [61]	—
MHCPRED	JenPep [19]	—
MMBPred	MHCBN [68]	—
MULTIPRED	See NetMHCpan and NetMHCIIpan	—
NetCTL	See NetMHC	—
NetMHC	Trained for 81 HLA alleles including HLA-A, -B, -C and –E, n/s	—
NetMHCcons	IEDB [61]	—
NetMHCII	Data by [19]	http://www.cbs.dtu.dk/suppl/immunology/NetMHCII-2.0.php
NetMHCIIpan	IEDB [61]	http://www.cbs.dtu.dk/suppl/immunology/NetMHCIIpan-3.0/
NetMHCpan	IEDB [61], IMGT/HLA database [1]	—
nHLAPred: ANNPred	MHCBN [68]	—
nHLAPred: ComPred	MHCBN [68], BIMAS [58]	http://crdd.osdd.net/raghava/nhlapred/matrix.html
PREDPEP	Pairwise potential table by Miyazawa and Jernigan [99]	—
ProPred	QMs by Sturniolo, Bono [95]	http://crdd.osdd.net/raghava/propred/page4.html
ProPred I	BIMAS [58] and matrices by Ruppert, Sidney [100] and Sidney, Southwood [101]	http://crdd.osdd.net/raghava/propred1/matrices/matrix.html

Name	Underlying database/data source	URL for matrices/training data
Rankpep	MHCPEP [102], SYFPEITHI [94], GenBank [103]	—
svmhc	MHCPEP [102], SYFPEITHI [94]	http://www.cs.cornell.edu/people/tj/svm_light/
SYFPEITHI	Published literature	—
TEPITOPEpan	n/s	http://datamining-iip.fudan.edu.cn/service/TEPITOPEpan/TEPITOPEpan.html

Abbr. n/s = not specified.

Table 2. Listing of the underlying databases/data sources for peptide binding prediction.

5. Analysis of naturally presented peptides

The analysis of the individual patient and cell-type-specific immunopeptidome can be realized through sequencing the HLA-bound peptides. It is imperative for all ongoing peptide studies and cellular therapies to find peptides that are (1) naturally presented by the distinct allele, (2) immunogenic for (at best) a public T-cell repertoire and (3) preferentially presented when different peptides are available. A study from Yaciuk et al. showed for example that the peptides isolated from HIV-infected T cells differ from predicted peptides and exhibit different T-cell reactions, factors that have to be considered in designing immunotherapies [46]. That information is only available after immunopeptidome analyses.

In the past, different methods have been applied to answer these questions comprehensively. There are two reliable methods to determine peptide sequences from selected HLA alleles. First, membrane-bound HLA molecules from recombinant single-antigen-presenting cells [47, 48] or from donor cells [49, 50] can be captured by affinity chromatographic methods and the bound peptides isolated and sequenced by mass spectrometry. Second, the most realizable method is the soluble HLA technology [16, 51]. Vectors encoding for soluble forms of HLA molecules (Exon 1–4) are transfected or lentivirally transduced into the cell line of choice. An optional recombinant tag (e.g., V5 tag) engineered at the C-terminus of the protein enables specific purification of the recombinant HLA molecule of choice without the challenge of contamination by cellular-self-HLA molecules. Both methods have been compared by Scull et al. [52] and indicated as an equivalent for the determination of allele-specific peptides. Furthermore, Badrinath et al. [10] could demonstrate that sHLA molecules associate during peptide acquisition with the loading complex as well. These results prove evidence that the use of sHLA technology for understanding allele-specific peptide-binding motifs, the prerequisite for updating peptide prediction databases, is the most time- and cost-efficient implementation.

For the development of tailor-made T-cell-based immunotherapeutic strategies, the identification of tumor-specific HLA ligands is imperative. The production of recombinant sHLA-expressing cells derived from various tissues of malignant origins would guide towards understanding immune dominance through peptide competition. One of the most innovative applications is the peptide fishing from tumor tissue. Immunological tolerance is mediated through T cells that

are primed in the thymus by self-peptides. Therefore, the comprehensive knowledge of the HLA immunopeptidome from diseased cells is fundamental for the development of efficient immuno-therapeutic strategies. The presentation of peptides depends on the health state of a patient. During infections, the expression of HLA molecules and thus peptide presentation, including presentation of self-peptides, is diminished through an immune escape mechanism of the invasive pathogen.

6. Peptide vaccination

The treatment of cancer represents a great challenge due to the fact that the vast majority of HLA-restricted peptides differs from tissue to tissue and is dependent on the tumor entity. For that reason, it becomes obvious how fundamentally important the knowledge of the tumor-specific peptidome is. For personalized cancer immunotherapies, the knowledge of naturally presented peptides [53] represents the exclusive possibility for therapeutical suc-cess. The analysis of the mutanome, the proteomic content of a diseased cell, includes the dis-covery of neo-antigens or post-translational-modified peptides and the avoidance of targeting self-antigens from healthy tissue. The results of such individual mutanomes might alter dur-ing the course of tumor progression [16]. In peptide vaccination trials, the use of multiple peptides in combination [54, 55] represents a useful method for targeting all MHC-presenting cells with the peptide of choice. Yet, since the cell type where the peptide(s) bind to cannot be traced, the rates of antitumor immune responses might differ from patient to patient and certain tumor cells where, for example, low MHC expression rates might remain undetected from the immune system. To achieve a comprehensive and precise analysis of presented tumor antigens, the method of antigen discovery and appropriate T-cell assay for knowledge of immunogenicity of the dedicated antigen for vaccination is the key factor [56, 57].

7. Conclusion

Peptide selection and presentation is an exquisite biological and immunological event. Every single peptide is a mirror of the health state of a distinct cell and determines the outcome of immune recognition and responses. For all cellular therapies, the knowledge of the HLA-subtype specific proteome is crucial for the utilization of ligand prediction tools, which have to be implemented where no experimental data are available, yet.

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