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Research paper

In silico identification of epitopes present in human heat shock proteins (HSPs) overexpressed by tumour cells

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ABSTRACT

Although many of heat shock proteins (HSPs) are crucial in homeostasis due to their role in maintaining cellular proteostasis by the integration of two pivotal processes—folding and degradation, several decades of cancer proteomics suggest that HSPs may improve cancer establishment and progression. Therefore, it is imperative to explore how these molecules impact patient outcomes and whether their interaction with the immune systems improves the protumour or antitumour environment. Here, using an immunoinformatics approach were investigated the best probable epitopes from ten HSPs (HSP90α, HSP90β, HSPA1A, HSPA1L, HSPA2, HSPA5, HSPA6, HSPB1, HSPB5 and HSP60/HSP10). To achieve this aim, antigenicity, immunogenicity (prediction of continuous and discontinuous B cell epitopes, binding peptides to HLA class I and HLA class II, and overlapping epitopes), analysis of conservancy and population coverage, and prediction of IgE epitopes were evaluated. According to the physicochemical properties used for their prediction (hydrophilicity, flexibility, accessibility and antigenicity propensity), ten continuous epitopes (one per HSPs) were considered as the best and also several regions of each molecule were identified as B discontinuous epitopes. Interestingly, peptides of HSP90β, HSPA2, HSPB1, and HSPB5 were predicted as both continuous and discontinuous B cell epitopes. For all the HSPs evaluated were identified potential overlapping epitopes ("NTFYSNKEI", "TTYSCVGVF", "TADRWRVSL", "VKHFSPEEL" and "CEFQDAYVL"). Moreover, these peptides were negative for IgE epitopes and showed a large coverage in the human population (HLA-A*02, HLA-B*15, HLA-C*03, and HLA-C*12). Taken together, these data indicate that such epitopes may activate both the humoral and cell-mediated response, and thus serve as therapeutic targets for cancer. However, it must be assessed their efficacy and safety *in vitro* and *in vivo* before their translation in clinical trials.

1. Introduction

Heat shock proteins (HSPs) may aid either to stop or improve cancer establishment and progression (Ciocca and Calderwood 2005). This dual role is related to the intrinsic characteristics of HSPs that enable them to bind provisionally to newly synthesized polypeptides to support their suitable folding, assembly, and intracellular trafficking. Moreover, under stress conditions, HSPs prevent protein aggregation and enhance their renaturation, in fact, such capacity to preserve the structure and promote protein-protein interaction made possible the emergence of their common name—molecular chaperones (Beckmann et al. 1990; Freeman and Yamamoto 2002). This group of molecules is induced by a variety of environmental stressors including heat shock, viral infection, oxidative stress, UV radiation, toxins, among others (Lindquist and Craig 1988; Georgopolis and Welch 1993), in extracellular and cell-associated compartments in bacteria, plants, and human, thereby indicating that HSPs are highly conserved in all kingdoms of life (Johnston et al. 1980; Key et al. 1981; Miller et al. 1982; Tilly et al. 1983; Bradwell and Craig 1984). Regarding their regulation, the major transcription factor associated with HSPs expression is the heat shock transcription factor one (HSF1), which ensure fast transcriptional activation in stress and equally precipitous switch-off after recovery. Furthermore, HSPs activity is mainly controlled by ATP and co-chaperones (Sorger and Pelham 1988; Edkins et al. 2017).

According to their molecular weight, HSPs can be classified within the 70 kDa and 90 kDa chaperone families, which have an N-terminal nucleotide binding domain (NBD) with ATPase activity (Subbarao et al. 2004; Wisniewska et al. 2010). On the other hand, small heat shock proteins (sHSPs) ranging from 15 kDa to 40 kDa and carry a highly conserved domain known as the α-crystalline domain (ACD) that

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represents their identification flag (Carra et al. 2017). This family, whose function is ATP independent, perform critical functions in multiple signaling pathways in mammals, for instance, αB-crystalline (HSPB5) and Hsp27 (HSPB1) are implicated in improve anti-apoptotic pathways in cancer cells (Bruey et al. 2000; Kamradt et al. 2001; McLemore et al. 2005).

Cancer is one of the most leading causes of death all over the world (Fortunato et al. 2017), and its progression and expansion towards other tissues is related to high expression of oncogenic molecules, genomic instability, altered cellular metabolism as well as toxic insults that stimulate cancer growth through survival pathways such as the heat shock response (HSR) pathway (Edkins et al. 2017), whereby is influenced gene transcription and the synthesis of new proteins. Likewise, increasing evidence has pointed out that HSPs are "moonlighting molecules" —that is, proteins that may carry out more than one function in the cytosol, on the plasma membrane, and in the extracellular space (Edkins et al. 2017). For the purpose of this research, we may group these functions in two pivotal activities: protumour and antitumour. The former includes protection against apoptosis, tumour cell growth and migration, therapy resistance, tumour cell cross-talk, and immune tolerance, while the latter involves tumour cell recognition, activation, and migration of immune cells and tumour cell death (Shevtsov et al. 2017). In this regard, current works have related elevated HSPs levels with the histopathological characteristics of several tumours including breast-, ovarian-, uterine-, esophageal-, gastric-, liver-, lung-, and prostate cancer wherein the main HSP families involved with the patient outcome are HSP70 (HSPA), HSPB1, HSPB5, HSP90 (HSPC), and HSP60/HSP10 (HSP60 kDa/HSP 10 kDa) (Ciocca and Calderwood 2005).

The use of the immune system to fight cancer has been focused of intense investigation over the last decades (Johnson et al. 2009; Lechner et al. 2011; Iwai et al. 2012; Nobuoka et al. 2013) wherein immunoinformatics techniques have allowed an accelerated growth of the design and study of algorithms for mapping potential B- and T cell epitopes (immunologically active regions of an immunogen) to achieve the development of new therapeutics that positively impact cancer immunotherapy at faster time and lower cost (Tomar and De 2010). In this regard, HSPs are promising tumour targets for cancer immunotherapy, however, until now the main therapeutics against overexpressed HSPs comprise small molecule inhibitors, protein aptamers and antisense oligonucleotides (Chatterjee and Burns 2017), and only a few approaches employ the immune response to attack tumours through the recognition of HSPs, such as the monoclonal antibody (mAb) cmHsp70.1, which binds to the extracellular motif—TKDNNLLGRFELSG (TDK) on membrane-bound HSP70 (Stangl et al. 2011). Currently, this mAb is tested in combination with chemoradiation therapy in a phase II trial involving non-small-cell lung carcinoma patients (NCT02118415). Other similar trials include several vaccines consisting of disease-specific epitopes and HSP70 DNA (NCT00027144; NCT00121173; NCT00030303; NCT02118415).

In this work, the identification of epitopes on molecular chaperones (HSPB1, HSPB5, HSP90, HSP70 and HSP60/10), which have been recognized previously as upregulated in several cancers (Ciocca and Calderwood 2005), was carried out using immunoinformatics tools to elucidate novel immunogenic determinants and provide clinically relevant potential therapeutic targets for cancer treatments and immunodiagnostics.

2. Methods

2.1. Protein sequence retrieval

Taking into account that the main HSP inhibitors are directed to the NBD in HSP70 and HSP90 (Chatterjee and Burns 2017), it was decided to study this region in these HSPs, as well as were selected two and five isoforms, respectively. On the other hand, the whole molecule from HSPB1, HSPB5, and HSP60/HSP10 was analyzed. The protein sequence from HSP90α, HSP90β, HSPA1A, HSPA1L, HSPA2, HSPA5, HSPA6, HSPB1, HSPB5 and HSP60/HSP10 was retrieved in FASTA format from Protein Data Bank (PBD) (accession Nos. 3T0Z, 1UYM, 3JXU, 3GDQ, 3I33, 3IUC, 3FE1, 2N3J, 3L1G and 4PJ1, respectively). The data acquisition of all HSPs evaluated is summarized in Table 1.

Table 1
Summary of data acquisition of all HSPs evaluated.

HSP	Isoform	PDB ID	Length (aa*)	Region Evaluated[a]	Molecular weight (KDa)
HSP90	alpha	3T0Z	228	NBD	29.71
	beta	1UYM	220	NBD	28.58
HSP70	HSPA1A	3JXU	409	NBD	52.51
	HSPA1L	3GDQ	408	NBD	52.26
	HSPA2	3I33	404	NBD	51.64
	HSPA5	3IUC	408	NBD	52.47
	HSPA6	3FE1	403	NBD	51.63
HSP27	B1	2N3J	98	EP	12.62
HSPB5	alpha	3L1G	96	EP	12.61
HSP60/HSP10		4PJ1	9408	EP	1242.69

[a] EP: entire protein.
* Amino acids.

2.2. Tumour antigenicity

Tumour antigenicity (capacity of HSPs to bind specifically to T cell receptors and membrane-bound or free antibodies [Abs]) for each probable epitope was corroborated on Vaxijen (www.ddg-pharmfac.net/vaxijen/) using the most accurate threshold (0.5) established by Doytchinova and Flower (2007).

2.3. Immunogenicity

The overall parameter was computed using EPITOPIA (http://epitopia.tau.ac.il/) that predict the immunogenic nature of a protein implementing a machine learning scheme to rank individual amino acids in a protein, according to their potential of eliciting a humoral immune response (Rubinstein et al. 2009). The input (PDB 3D-structure) of each HSP was analyzed according to its physicochemical and structural-geometrical properties. Immunogenic and solvent accessible residues in the input structure were visualized through JSmol, which express the immunogenicity score by a colour scale. Moreover, to obtain specific results according to B and T cell immune responses, several immunoinformatics tools—described in the next sections—were used to achieve this aim.

2.3.1. Prediction of epitopes for B lymphocytes

Amino acid sequences of HSPs were submitted to online servers to predict linear and conformational epitopes for B lymphocytes, using BCPRED (http://ailab.ist.psu.edu/bcpred/) and Ellipro (http://tools.iedb.org/ellipro/), respectively. In addition, with BCPred (http://crdd.osdd.net/raghava/bcepred/bcepred_submission.html) overlapping linear epitopes were chosen based on the following criteria: hydrophilicity, flexibility, accessibility and antigenicity propensity (threshold = 1 for each parameter). Prediction of conformational epitopes was performed at a minimum level of 0.8, and the maximum distance for residue clustering was defined as 6.0 Å. The main epitopes were visualized with JSmol to illustrate their 3D structure and position.

2.3.2. Prediction of HLA binding peptides

Prediction of binding peptides to human leukocyte antigen (HLA) class I and HLA class II were identified using several prediction tools on the Immune Epitope Database and Analysis Resource (IEDB-AR), a website of experimentally characterized immune epitopes (B and T cell epitopes) for several animal species including human, nonhuman

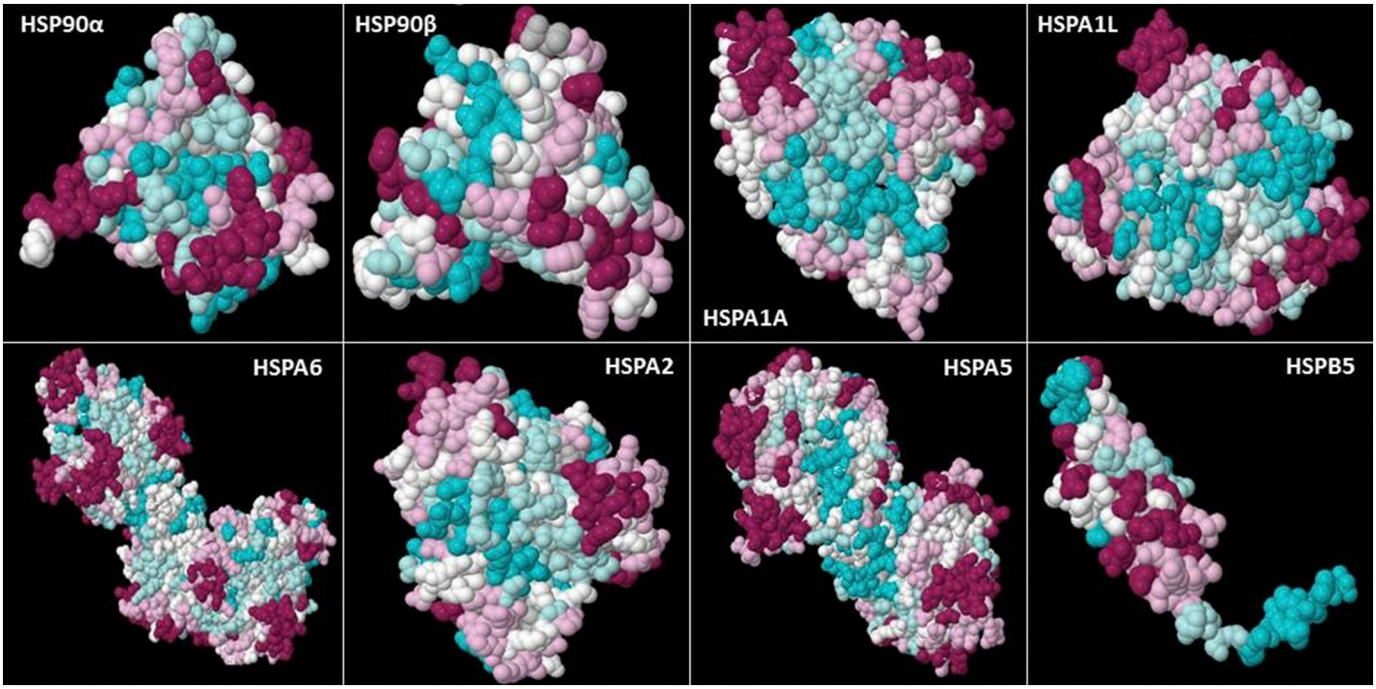

Fig. 1. Immunogenicity predicted by Epitopia web server for several HSPs studied. Colors indicate the immunogenicity score: low (turquoise), average (cyan, white, pink), and high (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

primates, mouse, among others (http://tools.immuneepitope.org/main/). The protein sequences in FASTA format were loaded on IEDB-AR and through the artificial neural network (ANN) method (Nielsen et al. 2003; Tenzer et al. 2005) the 9-mer binding peptides to HLA class I were predicted, while a combination of the average relative binding (ARB) matrix method and the stabilization matrix alignment method (SMM_align), altogether known as consensus method (Wang et al. 2008), were used to obtain the 15-mer binding peptides to HLA class II. These binding peptides to HLA class I and HLA class II were classified using as reference the half-maximal inhibitory concentration of a biological substance (IC50nM) wherein peptides with IC50 values < 50 nM are considered high affinity, < 500 nM intermediate affinity and < 5000 nM low affinity.

2.4. Prediction of overlapping epitopes for humoral and cell-mediated immunity

An epitope may induce a stronger humoral and cellular immune response than others by overlapping region of amino acid sequence that binds to antigen-specific membrane receptors on lymphocytes and secreted Abs, thereby enhancing the tumour attack (Salerno-Gonçalves and Sztein 2006). Therefore, it was imperative to evaluate the presence of such epitopes, using for this purpose the following databases: BCPred (for B cell epitopes), ANN (for 9-mer binding peptides to HLA class I), and ARB-SMM_align (for 15-mer binding peptides to HLA class II) in which was considered the IC_{50}nM to choose peptides with a high affinity for the HLA molecules.

2.5. Prediction of IgE epitopes and allergic sites

An allergic reaction involves the participation of a key immunoglobulin known as IgE whereby is induced the release of molecules that elicit irritation and inflammation when the interaction between this IgE and its specific antigen (allergen) occurs (Owen et al. 2013). Therefore, to determining the allergenicity of all predicted epitopes, a mapping of IgE epitopes was applied (www.imtech.res.in/raghava/algpred/).

2.6. Analysis of conservancy and population coverage

The HLA loci are highly polymorphic, that is, many alternative forms of the gene, or alleles, exist at each locus, and each set of these alleles is referred to as a haplotype (Owen et al. 2013). Therefore, promiscuous peptides (a peptide with the ability to bind to several HLA alleles) increasing human population coverage by which they would be attractive targets, for instance, in vaccine trials. In this regard, to avoid ethnically biased population coverage due to the exponential HLA polymorphism, through IEDB Analyses Resource (http://tools.iedb.org/population/) the fraction of individuals predicted to respond to a given epitope set on the basis of HLA genotypic frequencies and on the basis of MHC binding and/or T cell restriction data were calculate (Bui et al. 2006). The HLA allele genotypic frequencies are integrated on the website and were obtained from the Allele Frequency database (http://www.allelefrequencies.net/) that provides allele frequencies for 115 countries and 21 different ethnicities grouped into 16 different geographical areas. On the other hand, the degree of conservancy of an epitope within a given protein sequence set at a given identity level was analyzed in the same platform (http://tools.iedb.org/conservancy/). Conservancy and identity were defined as the fraction of protein sequences that contain the epitope and the degree of correspondence (similarity) between two sequences, respectively (Bui et al. 2007).

3. Results

3.1. Immunogenicity

This property was evaluated using EPITOPIA, which infers the immunogenic potential at the single amino acid site resolution computing an immunogenicity score from each solvent accessible residue. The HSP90 (HSP90α and HSP90β) and HSP70 (HSPA1A, HSPA1L, HSPA2, HSPA5, and HSPA6) isoforms, as well as HSPB5, showed a potential score of immunogenicity distributed along their surfaces, thereby designating probable B cell epitopes (Fig. 1). Unfortunately, due to the structural complexity of HSP60/HSP10 and HSPB1 was impossible to compute their overall immunogenicity. Although EPITOPIA may

Table 2
Best probable continuous B cell epitopes predicted from different HSPs using several parameters.

HSP	Epitopes[a]	Tumour antigenicity[b]	Hydrophilicity (average)	Flexibility (average)	Accessibility (average)	Antigenicity propensity (average)	Epitope conservancy	Allergenicity[c]
HSP90α	FVEKERDKEVSDDEAE	0.80	0.22	2.24	1.75	1.47	100%	Negative
HSP90β	PEEVHHGEEEVETFA	0.77	1.72	1.18	1.77	1.37	100%	
HSPA1A	IAYGLDRTGKGERN	1.27	2	2.33	2.1	0.53	100%	
HSPA1L	QVINEGGKPKV	1.42	0.33	1.58	1.35	1.35	100%	
HSPA2	IAYGLDKKGCAGGEKN	1.10	1.39	1.63	1.71	1.22	100%	
HSPA5	QSMDVGT	0.70	0.60	0.60	1.15	1.55	100%	
HSPA6	GRKFADTTVQSDMKH	0.60	1.69	0.74	1.83	1.19	100%	
HSP27	EMQLSSGVSEI	0.64	1.16	1.06	1.25	1.60	100%	
HSPB5	EVHGKHEERQDEHGF	0.75	0.96	1.50	1.50	1.6	100%	
HSP60/HSP10	SPYFINTSKGQKCEF	1.19	0.23	0.94	1.45	1.56	100%	

[a] Best residues are underlined.
[b] > 0.5 = positive.
[c] Mapping of IgE epitopes.

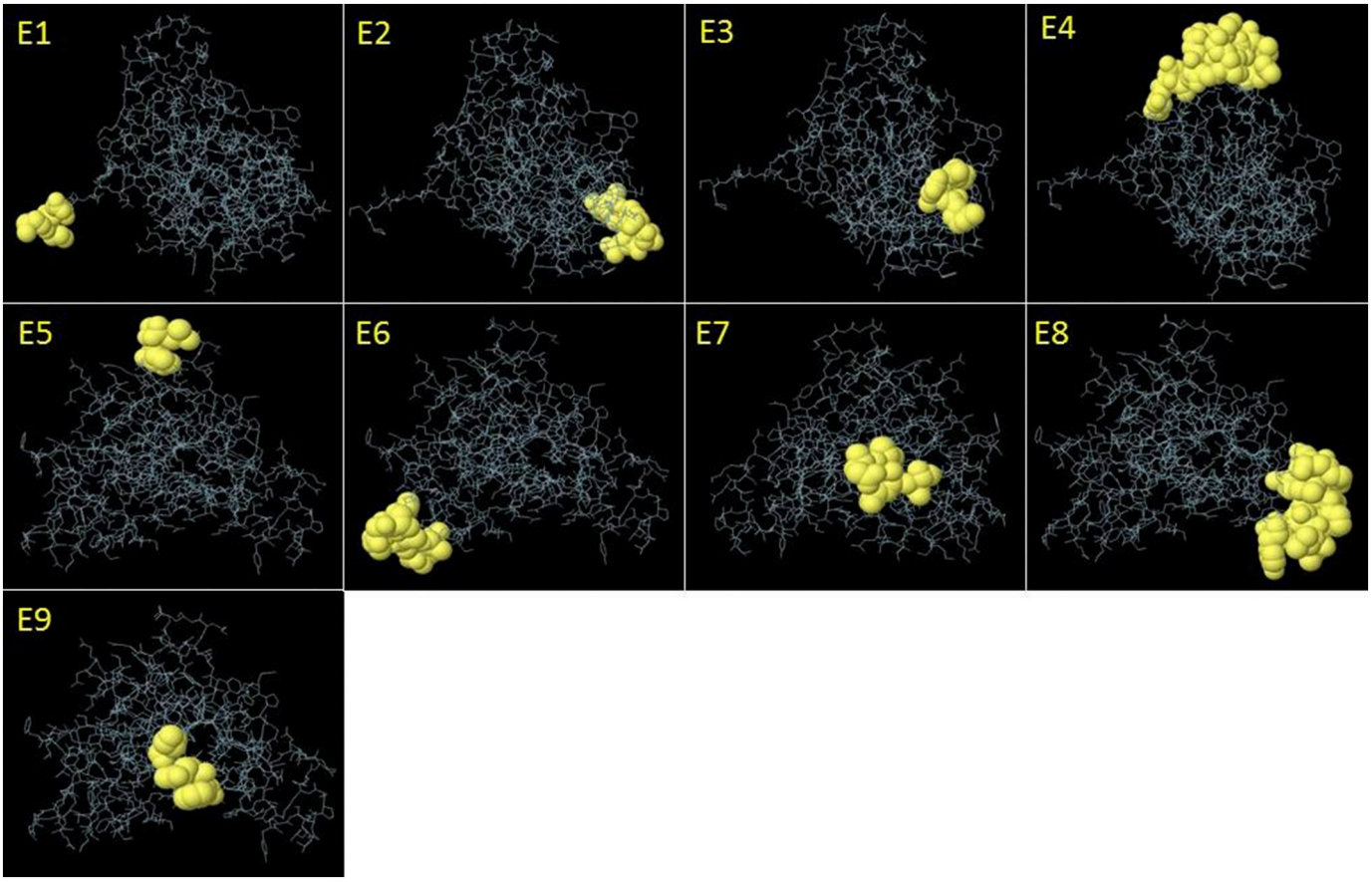

Fig. 2. Conformational B cell epitopes of HSP90α and HSP90β predicted from the 3D structure template under PDB ID 3T0Z and 1UYM, respectively. In the ball-and-stick model, yellow balls are the residues of predicted epitopes (HSP90α: E1 to E4, and HSP90β: E5 to E9) and white sticks are the structures for non-epitope and core residues. Each epitope is shown with predicted residues (abbreviated amino acids): E1, PME; E2, KAEAQAGA; E3, ERK; E4, RSLTDPSKLDSGKNDD; E5, PQE; E6, KAEALQAGA; E7, EVET; E8, YESLTDPSKLDSGKN; E9, KQF.

provide a general result in an attractive 3D structure for a protein 3D structure or a linear sequence, it is restricted to B cell epitopes and does not predict binding peptides to HLA class II and HLA class II. In addition, this software is unable to discriminate between continuous and conformational B cell epitopes that elicit the humoral response. To overcome this issue the protein sequences were analyzed on different web servers to determine continuous and conformational epitopes as well as binding peptides to HLA (class I and class II) molecules.

3.2. Identification of B cell epitopes

3.2.1. Identification of probable continuous B cell epitopes

Probable continuous B cell epitopes are listed in Table 2. They were chosen based on their physicochemical properties such as their surface accessibility, hydrophilicity, flexibility, antigenic and immunogenic properties, considering also their tumour antigenicity, the extent of conservancy and allergenicity. Thus, ten epitopes were considered as

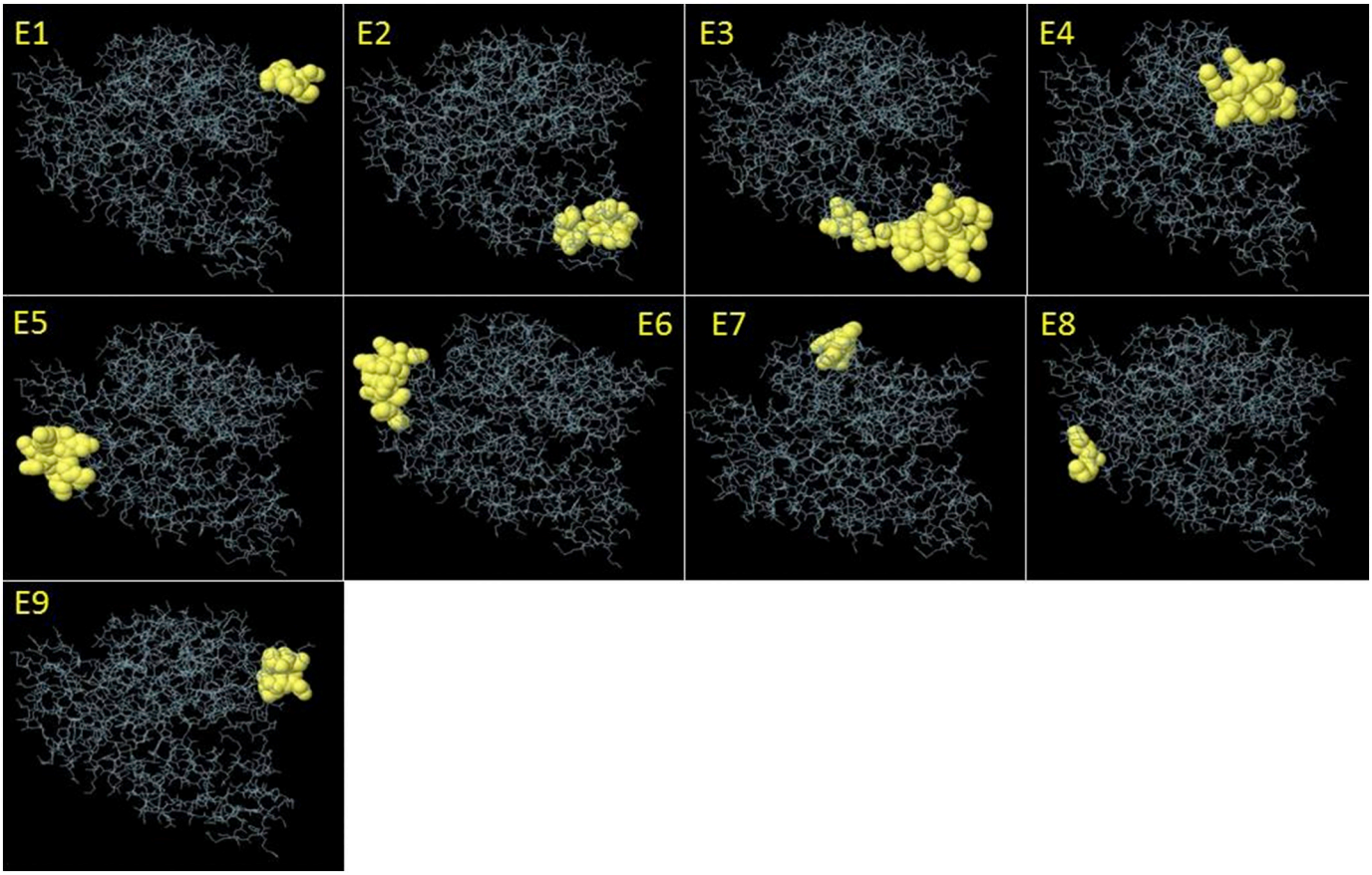

Fig. 3. Conformational B cell epitopes of HSPA1A predicted from the 3D structure template under PDB ID3JXU. In the ball-and-stick model, yellow balls are the residues of predicted epitopes (E1 to E9) and white sticks are the structures for non-epitope and core residues. Each epitope is shown with predicted residues (abbreviated amino acids): E1, GETK; E2, EDSGIDY; E3, EEFKRKHKKDISSTQFEAFTS; E4, KFGINDGDK; E5, RDAKLDKAQ; E6, TGKGERDDGH; E7, NGR; E8, LGYPV; E9, TDTEYK. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the best ones and all of them have shown the best scores of tumour antigenicity ranging from 0.60 to 1.42, however, the HSP70 (isoforms: HSPA1A, HSPA1L, and HSPA2) and HSP60/HSP10 families obtained the highest scores (Table 2).

3.2.2. Identification of conformational B cell epitopes

Amino acid sequences were analyzed on Ellipro server and conformational B cell epitopes were visualized through JSmol. From HSP90α, four residues were identified as potential B cell epitopes, while 5 residues were identified from HSP90β (Fig. 2). Regarding the HSP70 family, from HSPA1A, HSPA1L, HSPA2, HSPA5, and HSPA6 were found nine (Fig. 3), eleven (Fig. 4), ten (Fig. 5), five (Fig. 6) and two (Fig. 7) epitopes, respectively. Likewise, from HSPB1, only one epitope was identified and from HSPB5 three epitopes were recognized (Fig. 7). Interestingly, HSP60/HSP10 resulted in a whole target for Abs (Fig. 7). This is explained by its endosymbiotic origin that confer it a high structural and functional homology with the prokaryotic chaperonin GroEl-GroES (Nielsen et al. 1999). Therefore, HSP60/HSP10 not only may stimulate an adaptive immune response but also, perhaps, has pathogen-associated molecular patterns (PAMPs) that trigger the innate immune response through the activation of toll-like receptors (TLRs) or other pattern recognition receptors (PRRs).

Amino acid sequence (residue positions designated as superscript numerals) "EDSGIDY^{283,285,286,290,291,292,294}" was found only in conformational B cell epitopes from the HSPA1A and HSPA1L isoforms. On the other hand, amino acid sequence "EVET$^{16—19}$" was predicted as a continuous and conformational B cell epitopes from HSP90β. A similar result was obtained from HSPA2, HSPB27 and HSPB5 wherein both linear and nonlinear B cell epitopes were predicted in "CAGGEK$^{191—196}$", "MQLSSGVSEI$^{79—88}$" and "ERQDHGF^{105,107,108,109,111,112,113}" amino acid residues, respectively.

3.3. T cell epitope prediction

3.3.1. Identification of HLA class I- and HLA class II-binding peptides

The protein sequences in FASTA format were summited to IEDB-AR database for HLA class I-binding peptides prediction using a combination of proteasomal processing, TAP transport, and MHC binding to produce a general score for each peptide's intrinsic potential of being a T cell epitope. Likewise, these protein sequences were analyzed in the above software to predict HLA class II-binding peptides. Peptides were selected considering the following criteria: IC50 values < 50 nM, interaction of a peptide with several HLA class I molecules (at least six alleles) and the ability to overlapping with HLA class II-binding peptides. Thus, "FQAEIAQLM" from HSP90 (both isoforms), "NLYFQSMAK" from HSPA1A, "MATAKGIAI" from HSPA1L, "VAMNPTNTI" from HSPA2, "RVMEHFIKL" from HSPA5, "HAVITVPAY" from HSP6, "LTVEAPMPK" from HSPB1, "KVLGDVIEV" from HSPB5, and "FGADARALM" from HSP60/HSP10 showed the best result in population coverage, conservancy and allelic variation as well as they did not present IgE epitopes (Table 3).

3.4. Identification of overlapping epitopes for humoral and cell-mediated immunity

For all HSPs evaluated were identified potential overlapping epitopes, which promote the simultaneous enhancement of the cellular and humoral immunity. The epitope "NTFYSNKEI" was found in both

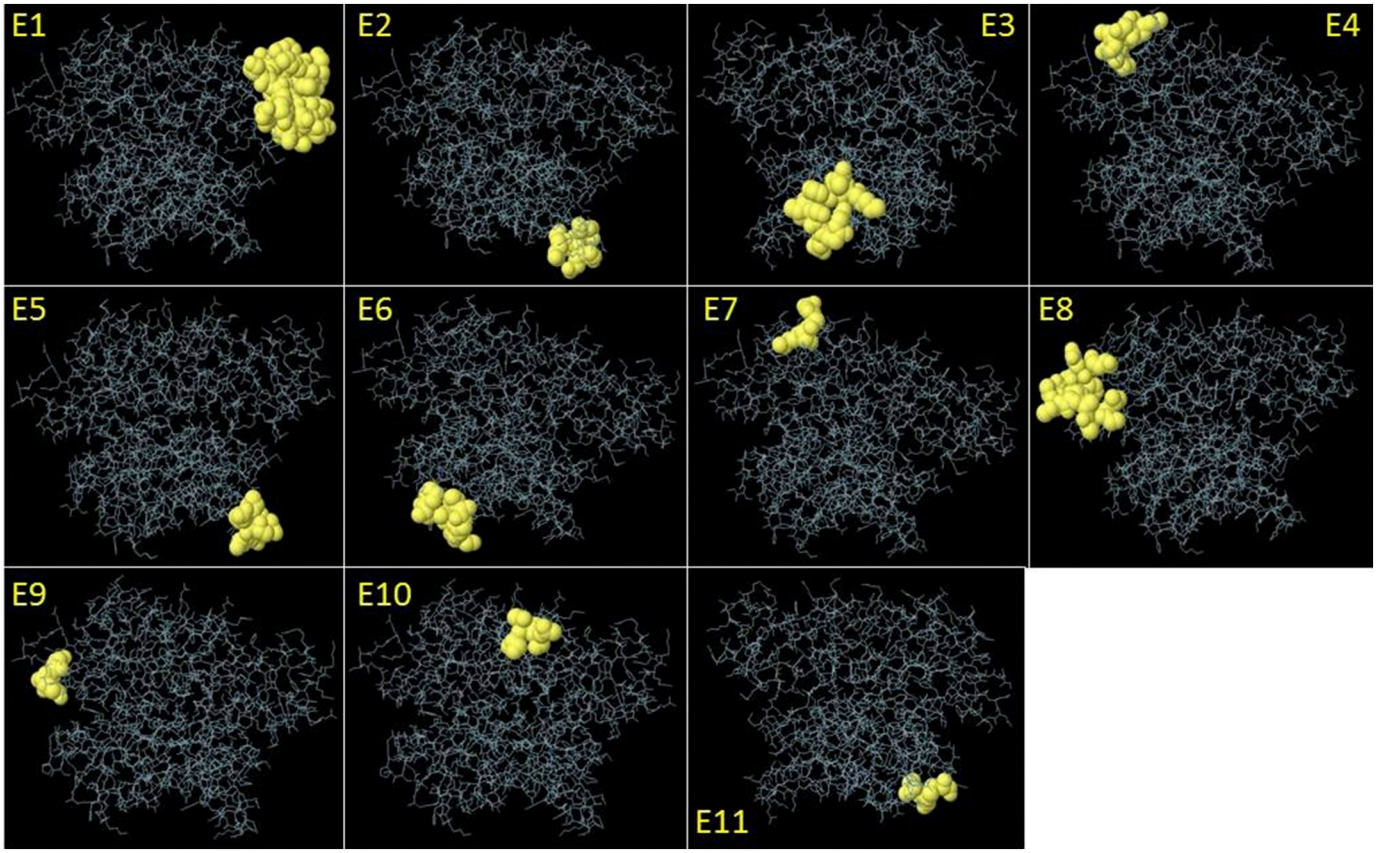

Fig. 4. Conformational B cell epitopes of HSPA1L predicted from the 3D structure template under PDB ID 3GDQ. In the ball-and-stick model, yellow balls are the residues of predicted epitopes (E1 to E10) and white sticks are the structures for non-epitope and core residues. Each epitope is shown with predicted residues (abbreviated amino acids): E1,TDTEYK; E2, GENK; E3, EDSGIDY; E4, KFNINEGG; E5, GEDDG; E6, ERDAKMDKAKH, E7; NGR; E8, LGHPV, E9, NFTS; E10, TDTEK; E11, GIM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

HSP90 isoforms, while for the HSP70 isoforms was recognized the epitope "TTYSCVGVF" as the most ubiquitous in this family. Furthermore, "TADRWRVSL", "VKHFSPEEL" and "CEFQDAYVL" were identified as overlapping epitopes from HSPB1, HSPB5, and HSP60/HSP10, respectively (Table 4).

4. Discussion

HSPs are involved in tumour cell proliferation, differentiation, invasion, metastasis, and death in different types of cancers (Lianos et al. 2015). Here, were selected several HSPs overexpressed in different human cancers to evaluate through immunoinformatics tools their ability to elicit a humoral and/or cell-mediated immune response against the tumour, thereby serving as possible targets for the development of novel immunotherapeutics.

Although EPITOPIA provided a general immunogenicity score for almost all HSPs studied, additional tools on the IEDB server were used to identify the most probable B cell epitopes. The main difference between continuous and conformational B cell epitopes is that the former are sequential residues along the polypeptide chain while the latter are nonsequential residues from segments of the chain brought together by the folded conformation of an antigen (Owen et al. 2013). The identification of such epitopes by means of immunoinformatics tools have a high impact on the development of new approaches to treat a range of pathologies, for instance, B cell epitopes of Emy162 protein of *Echinococcus multilocularis*, a parasite that causes a zoonotic infection resulting in a fatal chronic liver infestation, were predicted using BCPred and ABCPred (Arnaboldi et al. 2013). Therefore, epitopes predicted in this study, perhaps, have a similar potential for cancer treatment, of course, it will be needed future *in vitro* and *in vivo* studies to corroborate their clinical impact. According to the physicochemical properties evaluated (hydrophilicity, flexibility, accessibility and antigenicity propensity), ten continuous epitopes (one per HSPs) were considered as the best including "FVEKERDKEVSDDEAE$^{213—228}$" for HSP90α, "PEEVHHGEEEVETFA$^{1—15}$" for HSP90β, "IAYGLDRTGKGERN$^{203—216}$" for HSPA1A, "QVINEGGKPKV$^{117—127}$" for HSPA1L, "IAYGLDKKGCAGGEKN$^{201—216}$" for HSPA2, "QSMDVGT$^{21—27}$" for HSPA5, "GRKFADTTVQSDMKH$^{114—128}$" for HSPA6, "EMQLSSGVSEI$^{18—28}$" for HSPB1, "EVHGKHEERQDEHGF$^{52—66}$" for HSPB5, and "SPYFINTSKGQKCEF$^{227—241}$" for HSP60/HSP10 (Table 2). Also, the epitope residues underlined in Table 2 fulfilled with all physicochemical properties with the exception of hydrophobicity that was the criterion with the lowest value (0.97 on average). This is not a matter of concern because B cell epitopes on native proteins generally are composed by hydrophilic amino acids and tend to be topographically accessible to membrane-bound or free Abs (Owen et al. 2013). Indeed, the main current hydrophilic amino acids on HSPs epitopes were E, D, H, R, K, and Q, while the flexibility and accessibility reached high values (2.33 and 2.1 respectively) and passed the threshold level. Likewise, their sequences are enriched with enhancers of immunogenicity such as aromatic amino acids (Y and F) (Owen et al. 2013).

40 conformational epitopes were predicted, with a length from 3 to 7 amino acids (Fig. 2) to Fig. 7). Interestingly, the amino acid sequence "EVET$^{16—19}$", "CAGGEK$^{191—196}$", "MQLSSGVSEI$^{79—88}$" and "ERQDHGF^{105,107,108,109,111,112,113}" were recognized as linear and conformational B cell epitopes. This is due to that most of identified linear antigenic determinants form part of conformational B cell epitopes (Laver et al. 1990; Van Regenmortel 1996; Rubinstein et al. 2008). Nevertheless, several works (Janin and Chothia 1990; Kunik and Ofran 2013; Kringelum et al. 2013) have suggested that such amino

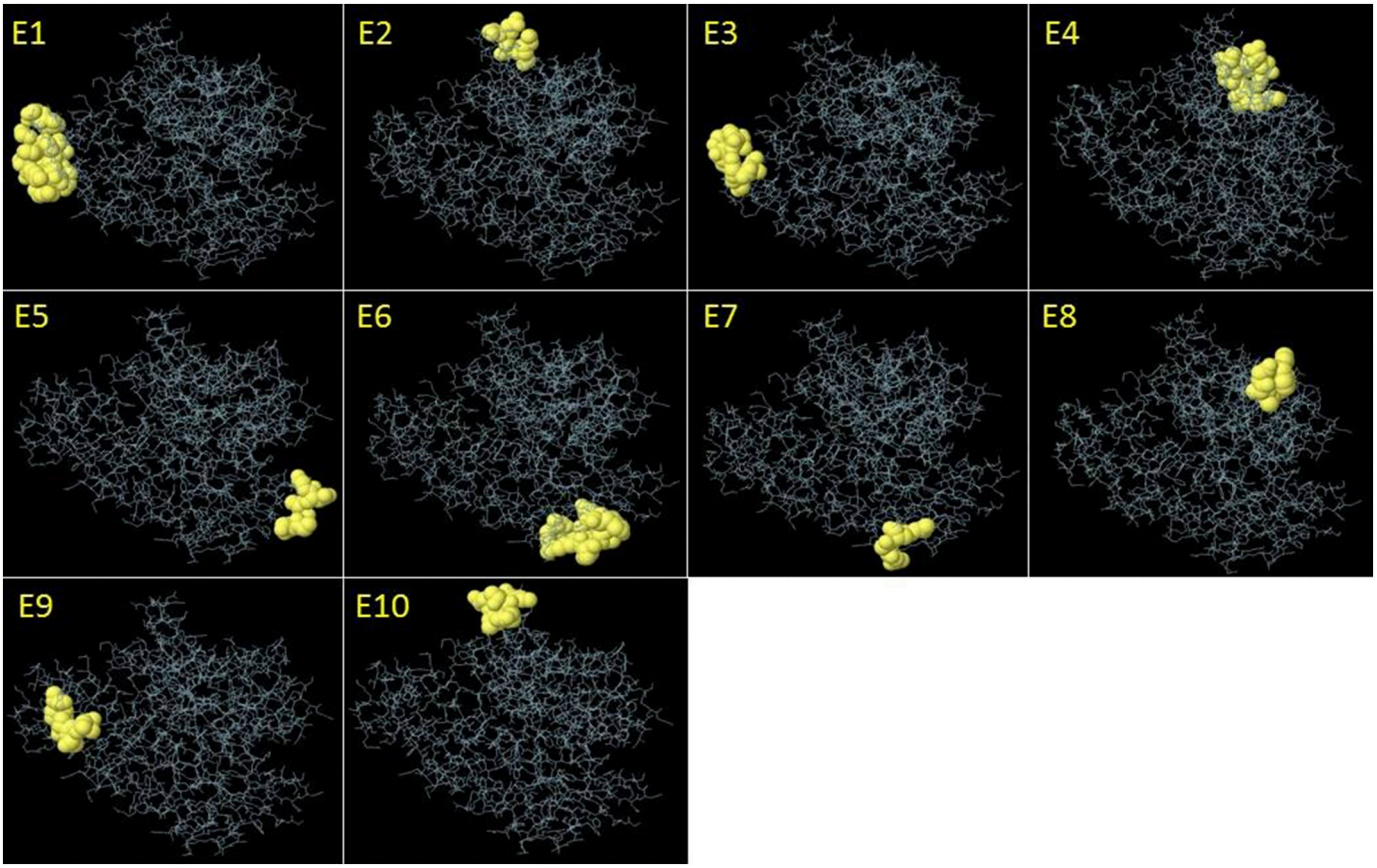

Fig. 5. Conformational B cell epitopes of HSPA2 predicted from the 3D structure template under PDB ID 3I33. In the ball-and-stick model, yellow balls are the residues of predicted epitopes (E1 to E10) and white sticks are the structures for non-epitope and core residues. Each epitope is shown with predicted residues (abbreviated amino acids): E1, EEFKRKHKKDIGPEDSYEGVDFYTS; E2, EGETK; E3, KFEVSEGG; E4, AKLDKGQ; E5, TDTEK; E6, MPLGGKV; E7, NGK; E8, GLKKGCAGGEKQ; E9, STQ; E10, EDG. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

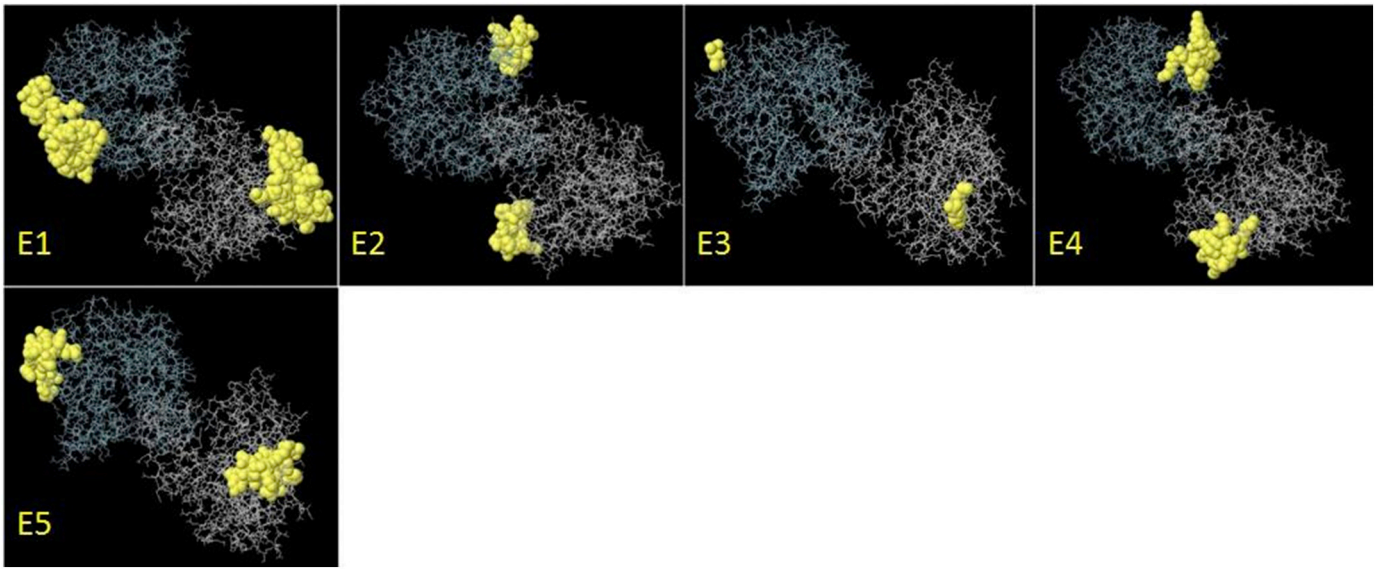

Fig. 6. Conformational B cell epitopes of HSPA5 (Homodimer) predicted from the 3D structure template under PDB ID3IUC. In the ball-and-stick model, yellow balls are the residues of predicted epitopes (E1 to E5) and white sticks are the structures for non-epitope and core residues. Each epitope is shown with predicted residues (abbreviated amino acids): E1, GDKREGEKNTIDNGVESDLKKSDID; E2, TPEGENDIGGGQTKT; E3, KNGR; E4, NGKEP; E5, GTVIEAYLGKKVTHTGLS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acid composition is indistinguishable between epitopes and non-epitopes. In this sense, we must consider not only epitope intrinsic properties but also the antigen-binding site on the Ab, such as the hypervariable regions or the complementarity-determining regions (CDRs), to refined immunoinformatics algorithms and avoid the expensive, laborious and time consuming of current methods for epitope

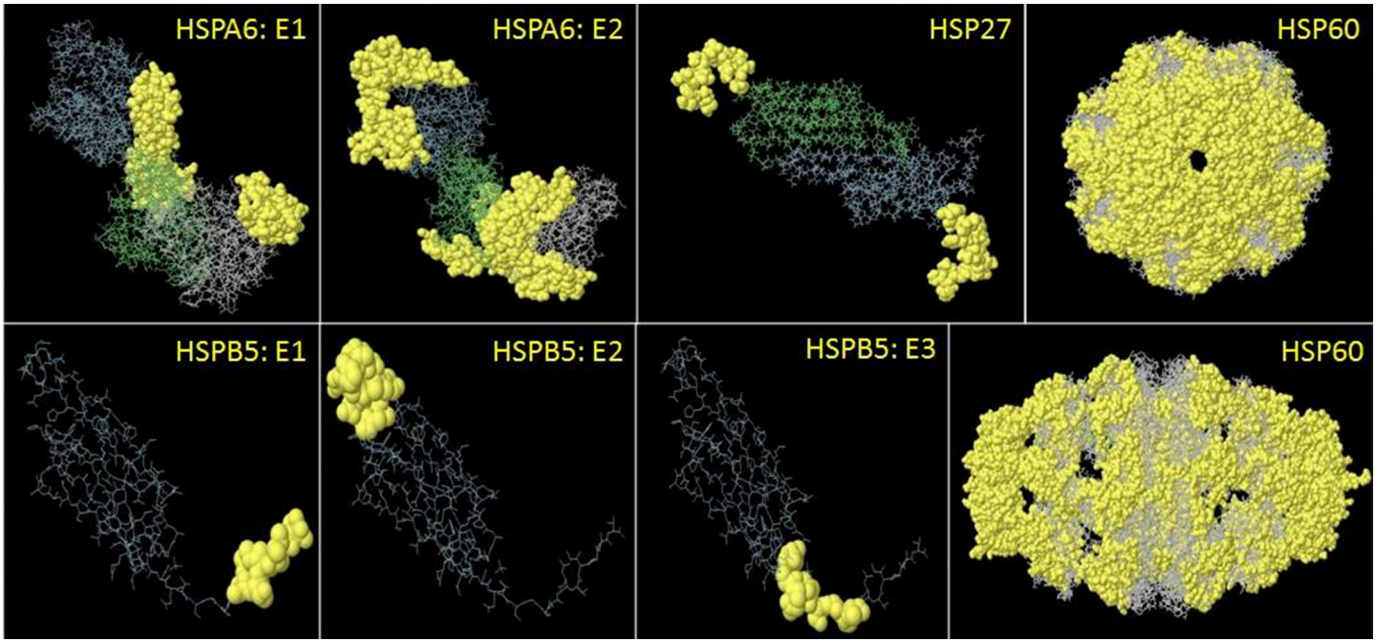

Fig. 7. Conformational B cell epitopes of HSPA6 (Homodimer), HSP27(Homodimer), HSPB5 and HSP60/HSP10 predicted from the 3D structure template under PDB ID3FE1, 2N3J, 3L1G, and 4PJ1, respectively. In the ball-and-stick model, yellow balls are the residues of predicted epitopes and white sticks are the structures for non-epitope and core residues. Each epitope is shown with predicted residues (abbreviated amino acids): E1, GLDRRGAGERNVLVLSIDAGVFEVKATALEPVEKALRDAKLDKAQIHDQDFFNGKEL; E2, AALNPHNKFAQMKWPFRVVSEGGRVCGEDKTRLVNHFMEEFRRKHGKDLSGNKRALRRLTLSSSTQATLEIDSLFEGVDFYTSITRARFEELCSDRKKLD for HSPA6; E1, MQLSSGVSEIRHTAD for HSP27; E1, RTIPIT; E2, ERQDHGFI; E3, VSGPE for HSPB5. In the case of HSP60/HSP10 the whole molecule resulted as a target for antibodies. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mapping (Sela-Culang et al. 2013; Potocnakova et al. 2016).

Antigen-presenting cells (APCs) load endogenous intracellular peptides onto the HLA class I molecules (HLA-A, —B and —C) and present these peptides to CD8+ T cells, as well as they regulate innate immune responses by interacting with killer cell immunoglobulin-like receptors (KIRs) expressed on natural killer (NK) cells (Parham 2005; Shiina et al. 2009), whereas the HLA class II molecules (HLA-DP, −DQ and -DR) bind peptides exclusively derived from exogenous proteins and engage with CD4+ T cells (Rehermann 2016). In this study, the HLA-binding peptides predicted, like "FQAEIAQLM" from HSP90 (both isoforms), "NLYFQSMAK" from HSPA1A, "MATAKGIAI" from HSPA1L, "VAMNPTNTI" from HSPA2, "RVMEHFIKL" from HSPA5, "HAVITVPAY" from HSP6, "LTVEAPMPK" from HSPB1, "KVLGDVIEV" from HSPB5, and "FGADARALM" from HSP60/HSP10, were found to interact with the maximum number of HLA class I and HLA class II alleles established for the analysis (Table 3). Moreover, 9-mer peptides ("NTFYSNKEI" from both HSP90 isoforms, "TTYSCVGVF" from all HSP70 isoforms, "TADRWRVSL" from HSPB1, "VKHFSPEEL" from HSPB5 and "CEFQDAYVL" from HSP60/HSP10) were compared with continuous B cell epitopes resulting in overlapping, thereby indicating that they possibly have the potential to induce simultaneously both humoral- and cell-mediated immune response. Furthermore, the main antigen-presenting alleles were HLA-A*02, HLA-B*15, HLA-C*03 and HLA-C*12 (Table 3 and Table 4). Due to its high prevalence, the HLA-A*02 allele could be an important molecule for cancer immunotherapy in most population worldwide, regardless race and ethnicity (Gonzalez-Galarza et al. 2011), for instance, the HLA-A*02:01 subtype is highly represented in human settlements in Europe, Asia, North America, South/Central America, Oceania, and Sub-Saharan Africa (Sidney et al. 2001; Castelli et al. 2005; El-Awar et al. 2007; Pekiner et al. 2013). In addition, the HLA-B*15 allele is also highly expressed in Costa Ricans, non-Hispanic African Americans, Colombian black ethnic group, South Africans, Cameroonians, as well as Bangladeshi and Japanese population, among others (Middleton et al. 2000; Samandary et al. 2014). In fact, as a result of their high frequency, these allelic groups (HLA-A*02 and HLA-B*15) have been considered for the development of a universal CD8+ T cell epitope-based vaccine against herpes simplex virus infections (Samandary et al. 2014). On the other hand, the HLA-C*03 allele has a strong linkage disequilibrium with HLA-B*40 or B*15 and occasionally it shares sequence homology with human HLA-A and HLA-B molecules by which it is difficult to distinguish between HLA-C from HLA-B restricted responses.

Previous works (Yamada et al. 1999; Takiguchi et al. 2000) demonstrate that 9-mer peptides bind with stronger affinity to HLA class I molecules than larger or shorter peptides and also containing specifics residues that improve HLA-binding. Almost all 9-mer peptides examined to date contain a carboxyl-terminal anchor (hydrophobic residues) that enhances the interaction bweteen the peptide and the groove of the HLA molecule (Owen et al. 2013). This pattern did not change in almost all HLA class I-binding peptides predicted in this research, in which the majority of anchor residues were hydrophobic (*e.g.*, L, V, and I) (Table 3 and Table 4).

Even though these and other peptides may be used in immunotherapy is probable that cancer cells do not present them onto HLA molecules because of alterations in HLA expression are a frequent and early event in carcinogenesis (Campoli and Ferrone 2008; Ferns et al. 2016). Thus, efforts to treat cancer through the induction of a CD8+ T cell response will be unsuccessful in tumours with negative or deficient HLA-I expression. Under these circumstances, HLA status on the tumour cell surface must first be confirmed to determine the suitability of an immunotherapy treatment based on T cell activation (Zhou and Levitsky 2012). In addition, although epitopes predicted in this study were negative for IgE epitopes and had a high potential to combine with the final products of the humoral and/or cell-mediated immune responses, which may induce a strong immune response against tumour cells, is imperative to evaluate their efficacy and safety by biological experimentation before translated them in human clinical trials.

Table 3
High-affinity (IC50 of < 50 nM) binding peptides to HLA class I and HLA class II from different HSPs.

HSP	HLA-I peptides	Interacting HLA-I alleles	Overlapping HLA-II peptides	Interacting HLA-II alleles	Population coverage	Conservancy analysis	Allergenicity
HSP90a	FQAEIAQLM	HLA-A*02:01	TFAFQAEIAQLMSLI	HLA-DRB1*04:01	80.7%	100% in both HSP90 isoforms	Negative
		HLA-A*02:06	ETFAFQAEIAQLMSL	HLA-DRB1*07:01			
		HLA-B*15:01	VETFAFQAEIAQLMS	HLA-DRB1*15:01			
		HLA-B*15:25	EVETFAFQAEIAQLM	HLA-DRB3*01:01			
		HLA-B*39:01	FAFQAEIAQLMSLII	HLA-DRB3*02:02			
		HLA-C*03:02	AFQAEIAQLMSLIIN				
		HLA-C*02:02	FQAEIAQLMSLIINT				
		HLA-C*02:09					
		HLA-C*03:03					
		HLA-C*03:04					
		HLA-C*12:02					
		HLA-C*12:03					
HSPA1A	NLYFQSMAK	HLA-A*03:01	TENLYFQSMAKAAAI	HLA-DRB1*01:01	53.16%	100%	Negative
		HLA-A*11:01	NLYFQSMAKAAAIGI	HLA-DRB1*09:01			
		HLA-A*68:01	GTENLYFQSMAKAAA	HLA-DRB1*11:01			
		HLA-C*03:02	ENLYFQSMAKAAAIG				
		HLA-C*03:03					
		HLA-C*03:04					
HSPA1L	MATAKGIAI	HLA-C*02:02	NLYFQSMATAKGIAI	HLA-DRB1*04:01	43.73%	100%	Negative
		HLA-C*03:02	LYFQSMATAKGIAIG	HLA-DRB1*09:01			
		HLA-C*03:03	YFQSMATAKGIAIGI	HLA-DRB3*02:02			
		HLA-C*03:04	FQSMATAKGIAIGID				
		HLA-C*08:01					
		HLA-C*12:03					
HSPA2	VAMNPTNTI	HLA-C*02:02	AKNQVAMNPTNTIFD	HLA-DRB1*03:01	66.25%	100%	Negative
		HLA-C*02:09	QVAMNPTNTIFDAKR	HLA-DRB1*04:01			
		HLA-C*03:02	VAMNPTNTIFDAKRL	HLA-DRB1*08:02			
		HLA-C*03:03	DAAKNQVAMNPTNTI	HLA-DRB1*13:02			
		HLA-C*03:04	NQVAMNPTNTIFDAK	HLA-DRB3*01:01			
		HLA-C*08:01	AAKNQVAMNPTNTIF				
		HLA-C*12:02	KNQVAMNPTNTIFDA				
		HLA-C*12:03					
HSPA5	RVMEHFIKL	HLA-A*02:01	DFDQRVMEHFIKLYK	HLA-DRB1*08:02	75.30%	100%	Negative
		HLA-A*02:06	DQRVMEHFIKLYKKK	HLA-DRB1*11:01			
		HLA-A*30:01	FDQRVMEHFIKLYKK	HLA-DRB1*12:01			
		HLA-B*08:01	RVMEHFIKLYKKKTG	HLA-DRB1*15:01			
		HLA-B*15:25	GEDFDQRVMEHFIKL	HLA-DRB3*01:01			
		HLA-C*03:02	EDFDQRVMEHFIKLY	HLA-DRB5*01:01			
		HLA-C*03:04	QRVMEHFIKLYKKKT				
		HLA-C*12:03	EDFDQRVMEHFIKLY				
HSPA6	HAVITVPAY	HLA-A*30:02	PVKHAVITVPAYFND	HLA-DRB1*04:05	51.35%	100%	Negative
		HLA-B*15:01	VKHAVITVPAYFNDS	HLA-DRB1*07:01			
		HLA-B*15:02	GQPVKHAVITVPAYF	HLA-DRB1*08:02			
		HLA-B*15:25	QPVKHAVITVPAYFN	HLA-DRB1*12:01			
		HLA-B*35:01	KHAVITVPAYFNDSQ	HLA-DRB1*15:01			
		HLA-C*03:02	HAVITVPAYFNDSQR	HLA-DRB3*01:01			
		HLA-C*12:03		HLA-DRB5*01:01			
HSPB1	LTVEAPMPK	HLA-A*03:01	EGTLTVEAPMPKLAT	HLA-DRB1*03:01	49.76%	100%	Negative
		HLA-A*11:01	PEGTLTVEAPMPKLA	HLA-DRB5*01:01			
		HLA-A*30:01	GTLTVEAPMPKLATQ				
		HLA-A*30:02	SPEGTLTVEAPMPKL				
		HLA-A*68:01	TLTVEAPMPKLATQS				
		HLA-B*15:01	LSPEGTLTVEAPMPK				
HSPB5	KVLGDVIEV	HLA-A*02:01	EELKVKVLGDVIEVH	HLA-DRB1*03:01	75.85%	100%	Negative
		HLA-A*02:06	ELKVKVLGDVIEVHG	HLA-DRB1*08:02			
		HLA-A*30:01	LKVKVLGDVIEVHGK				
		HLA-A*68:02	KVKVLGDVIEVHGKH				
		HLA-B*58:01	VKVLGDVIEVHGKHE				
		HLA-C*02:02	PEELKVKVLGDVIEV				
		HLA-C*02:09					
		HLA-C*03:02					
		HLA-C*03:03					
		HLA-C*03:04					
		HLA-C*12:03					
		HLA-C*12:02					
HSP60/HSP10	FGADARALM	HLA-B*35:01	KDVKFGADARALMLQ	HLA-DRB1*08:02	54.03%	100%	Negative
		HLA-C*02:02	DVKFGADARALMLQG	HLA-DRB1*09:01			
		HLA-C*02:09	AKDVKFGADARALML	HLA-DRB3*01:01			
		HLA-C*03:02	VKFGADARALMLQGV	HLA-DRB3*02:02			
		HLA-C*03:03	FGADARALMLQGVDL	HLA-DRB4*01:01			
		HLA-C*03:04	SAKDVKFGADARALM				
		HLA-C*08:01	KFGADARALMLQGVD				
		HLA-C*12:03	KDVKFGADARALMLQ				

Table 4
Potential overlapping epitopes for humoral and cell-mediated immunity.

HSP	HLA-I peptides	Interacting HLA-I alleles	Overlapping HLA-II peptides	Interacting HLA-II alleles	Potential B cell epitopes	Population coverage	Conservancy analysis	Allergenicity
HSP90	NTFYSNKEI	HLA-A*30:01 HLA-A*68:02 HLA-C*02:02 HLA-C*02:09 HLA-C*03:02 HLA-C*03:03 HLA-C*03:04 HLA-C*12:03 HLA-C*12:02 HLA-C*14:02	QLMSLIINTFYSNKE LMSLIINTFYSNKEI SLIINTFYSNKEIFL MSLIINTFYSNKEIF LIINTFYSNKEIFLR IINTFYSNKEIFLRE INTFYSNKEIFLREL	HLA-DRB1*04:01 HLA-DRB1*04:05 HLA-DRB1*07:01 HLA-DRB1*12:01 HLA-DRB1*15:01 HLA-DRB5*01:01 HLA-DRB5*01:01	NTFYSNKEIFL	74.58%	100% in both HSP90 isoforms	Negative
HSP70	TTYSCVGVF	HLA-A*02:06 HLA-A*26:01 HLA-A*29:02 HLA-A*68:02 HLA-B*15:01 HLA-B*15:02 HLA-B*15:25 HLA-B*35:01 HLA-B*58:01 HLA-C*02:02 HLA-C*02:09 HLA-C*03:02 HLA-C*03:03 HLA-C*03:04 HLA-C*12:02 HLA-C*12:03 HLA-C*14:02	AAIGIDLGTTYSCVG AIGIDLGTTYSCVGV IGIDLGTTYSCVGVF TYSCVGVFQHGKVEI TTYSCVGVFQHGKVE	HLA-DRB1*03:01 HLA-DRB5*01:01	DLGTTYSCVG	69.67%	100% among all HPS70 isoforms evaluated	Negative
HSPB1	TADRWRVSL	HLA-B*39:01 HLA-C*03:02 HLA-C*03:03 HLA-C*03:04 HLA-C*05:01 HLA-C*08:01 HLA-C*08:02 HLA-C*12:03 HLA-C*14:02	TADRWRVSLDVNHFA SSGVSEIRHTADRWR EIRHTADRWRVSLDV IRHTADRWRVSLDVN HTADRWRVSLDVNHF	HLA-DRB1*03:01 HLA-DRB1*04:01	QLSSGVSEIRHTADRWR	63.25%	100%	Negative
HSPB5	VKHFSPEEL	HLA-B*39:01 HLA-C*03:02 HLA-C*07:02 HLA-C*12:02 HLA-C*12:03 HLA-C*14:02	FSVNLDVKHFSPEEL NLDVKHFSPEELKVK VNLDVKHFSPEELKV LDVKHFSPEELKVKV SVNLDVKHFSPEELK DVKHFSPEELKVKVL VKHFSPEELKVKVLG KHFSPEELKVKVLGD	HLA-DRB1*03:01 HLA-DRB1*04:05 HLA-DRB1*07:01 HLA-DRB1*09:01 HLA-DRB1*11:01 HLA-DRB3*01:01 HLA-DRB5*01:01	DVKHFSPEELK	69.88%	100%	Negative
HSP60/HSP10	CEFQDAYVL	HLA-B*13:01 HLA-B*18:01 HLA-B*37:01 HLA-B*38:01 HLA-B*40:01 HLA-B*40:02 HLA-B*44:02 HLA-B*44:03 HLA-B*49:01 HLA-B*50:01 HLA-B*52:01	CEFQDAYVLLSEKKI EFQDAYVLLSEKKIS KCEFQDAYVLLSEKK GQKCEFQDAYVLLSE	HLA-DRB1*03:01 HLA-DRB1*04:01 HLA-DRB1*11:01 HLA-DRB5*01:01	INTSKGQKCEFQDA	63.28%	100%	Negative

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References

Arnaboldi, P.M., Seedarnee, R., Sambir, M., Callister, S.M., Imparato, J.A., Dattwyler, R.J., 2013. Outer surface protein C peptide derived from BorreliaburgdorferiSensuStricto as a target for serodiagnosis of early lyme disease. Clin. Vaccine Immunol. 20, 474–481. https://doi.org/10.1128/CVI.00608-12.
Beckmann, R.P., Mizzen, L.A., Welch, W.J., 1990. Interaction of HSP70 with newly

synthesized proteins; implications for protein folding and assembly. Science 248, 850–854. https://doi.org/10.1126/science.2188360.
Bruey, J.M., Ducasse, C., Bonniaud, P., Ravagnan, L., Susin, S.A., Diaz-Latoud, C., Gurbuxani, S., Arrigo, A.P., Kroemer, G., Solary, E., Garrido, C., 2000. Hsp27 negatively regulates cell death by interacting with cytochrome c. Nat. Cell Biol. 2, 645−652. https://doi.org/10.1038/35023595.
Bui, H.H., Sidney, J., Dinh, K., Southwood, S., Newman, M.J., Sette, A., 2006. Predicting population coverage of T-cell epitope-based diagnostics and vaccines. BMC Bioinform. 7, 153. https://doi.org/10.1186/1471-2105-7-153.
Bui, H.H., Sidney, J., Li, W., Fusseder, N., Sette, A., 2007. Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines. BMC Bioinform. 8, 361. https://doi.org/10.1186/1471-2105-8-361.
Campoli, M., Ferrone, S., 2008. HLA antigen changes in malignant cells: epigenetic mechanisms and biologic significance. Oncogene 27, 5869–5885. https://doi.org/10.1038/onc.2008.273.
Carra, S., Alberti, S., Arrigo, P.A., Benesch, J.L., Benjamin, I.J., Boelens, W., Bartelt-Kirbach, B., Brundel, B.J.J.M., Buchner, J., Bukau, B., Carver, J., Ecroyd, H., Emanuelsson, C., Finet, S., Golenhofen, N., Goloubinoff, P., Gusev, N., Haslbeck, M., Hightower, L., Kampinga, H.H., Klevit, R.E., Liberek, K., Mchaourab, H.S., McMenimen, K., Poletti, A., Quinlan, R., Strelkov, S., Toth, M., Vierling, E., Tanguay, R.M., 2017. The growing world of small heat shock proteins: from structure to functions. Cell Stress Chaperones 22, 601–611. https://doi.org/10.1007/s12192-017-0787-8.
Castelli, E.C., Gil, D.S., Veiga, L.C., de Camargo, J.L., 2005. Typing class I HLA-A gene using a nested PCR-RFLP procedure. Braz. J. Med. Biol. Res. 38, 837. https://doi.org/10.1590/S0100-879X2005000600004.
Chatterjee, S., Burns, T.F., 2017. Targeting heat shock proteins in cancer: a promising therapeutic approach. Int. J. Mol. Sci. 18, 1978. https://doi.org/10.3390/ijms18091978.
Ciocca, D.R., Calderwood, S.K., 2005. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 10, 86–103. https://doi.org/10.1379/CSC-99r.1.
Doytchinova, I.A., Flower, D.R., 2007. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinform. 8, 4. https://doi.org/10.1186/1471-2105-8-4.
Edkins, A.L., Price, J.T., Pockley, A.G., Blatch, G.L., 2017. Heat shock proteins as modulators and therapeutic targets of chronic disease: an integrated perspective. Phil. Trans. R. Soc. B. 373, 20160521. https://doi.org/10.1098/rstb.2016.0521.
El-Awar, N., Lee, J.H., Tarsitani, C., Terasaki, P.I., 2007. HLA class I epitopes: recognition of binding sites by mAbs or eluted alloantibody confirmed with single recombinant antigens. Hum. Immunol. 68, 170. https://doi.org/10.1016/j.humimm.2006.11.006.
Ferns, D.M., Heeren, A.M., Samuels, S., Bleeker, M.C., de Gruijl, T.D., Kenter, G.G., Jordanova, E.S., 2016. Classical and non-classical HLA class I aberrations in primary cervical squamous-and adenocarcinomas and paired lymph node metastases. J. Immunother. Cancer 4, 78. https://doi.org/10.1186/s40425-016-0184-3.
Fortunato, A., Boddy, A., Mallo, D., Aktipis, A., Maley, C.C., Pepper, J.W., 2017. Natural selection in Cancer biology: from molecular snowflakes to trait hallmarks. Cold Spring HarbPerspect Med. 7, a029652. https://doi.org/10.1101/cshperspect.a029652.
Freeman, B.C., Yamamoto, K.R., 2002. Disassembly of transcriptional regulatory complexes by molecular chaperones. Science 296, 2232–2235. https://doi.org/10.1126/science.1073051.
Georgopolis, C., Welch, W.J., 1993. Role of the major heat shock proteins as molecular chaperones. Ann. Rev. Cell Biol. 9, 601–634. https://doi.org/10.1146/annurev.cb.09.110193.003125.
Gonzalez-Galarza, F.F., Christmas, S., Middleton, D., Jones, A., 2011. Allele frequency net: a database and online repository for immune gene frequencies in worldwide populations. Nucl. Acid Res. 39, D913. https://doi.org/10.1093/nar/gkq1128.
Iwai, K., Soejima, K., Kudoh, S., Umezato, Y., Kaneko, T., Yoshimori, K., Tokuda, H., Yamaguchi, T., Mizoo, A., Setoguchi, Y., Kamigaki, T., Fujimoto, K., Goto, S., 2012. Extended survival observed in adoptive activated T lymphocyte immunotherapy for advanced lung cancer: results of a multicenter historical cohort study. Cancer ImmunolImmunother 61, 1781–1790. https://doi.org/10.1007/s00262-012-1226-4.
Janin, J., Chothia, C., 1990. The structure of protein-protein recognition sites. J. Biol. Chem. 265, 16027–16030.
Johnson, B.A., Baban, B., Mellor, A.L., 2009. Targeting the immunoregulatoryindoleamine 2,3dioxygenase pathway in immunotherapy. Immunotherapy 1, 645–661. https://doi.org/10.2217/IMT.09.21.
Johnston, D., Oppermann, H., Jackson, J., Levinson, W., 1980. Induction of four proteins in chick embryo cells by sodium arsenite. J. Biol. Chem. 255, 6975–6980.
Kamradt, M.C., Chen, F., Cryns, V.L., 2001. The small heat shock protein αB-Crystallin negatively regulates cytochrome candCaspase-8-dependent activation of Caspase-3 by inhibiting its Autoproteolytic maturation. J. Biol. Chem. 276, 16059–16063. https://doi.org/10.1074/jbc.C100107200.
Key, J.L., Lin, C.Y., Chen, Y.M., 1981. Heat shock proteins of higher plants. Proc. Natl. Acad. Sci. U. S. A. 78, 3526–3530.
Kringelum, J.V., Nielsen, M., Padkjær, S.B., Lund, O., 2013. Structural analysis of B-cell epitopes in antibody: protein complexes. MolImmunol 53, 24–3410. https://doi.org/10.1016/j.molimm.2012.06.001.
Kunik, V., Ofran, Y., 2013. The indistinguishability of epitopes from protein surface is explained by the distinct binding preferences of each of the six antigen-binding loops. Protein Eng. Des. Sel. 26, 599–609. https://doi.org/10.1093/protein/gzt027.
Laver, W.G., Air, G.M., Webster, R.G., Smith-Gill, S.J., 1990. Epitopes on protein antigens: misconceptions and realities. Cell 61, 553–556. https://doi.org/10.1016/0092-8674(90)90464-P.
Lechner, M.G., Russell, S.M., Bass, R.S., Epstein, A.L., 2011. Chemokines, costimulatory molecules and fusion proteins for the immunotherapy of solid tumors. Immunotherapy 3, 1317–1340. https://doi.org/10.2217/imt.11.115.
Lianos, G.D., Alexiou, G.A., Mangano, A., Mangano, A., Rausei, S., Boni, L., Dionigi, G., Roukos, D.H., 2015. The role of heat shock proteins in cancer. Cancer Lett. 360, 114–118. https://doi.org/10.1016/j.canlet.2015.02.026.
Lindquist, S., Craig, E.A., 1988. The heat shock proteins. Ann. Rev. Genet. 22, 631–637. https://doi.org/10.1146/annurev.ge.22.120188.003215.
McLemore, E.C., Tessier, D.J., Thresher, J., Komalavilas, P., Brophy, C.M., 2005. Role of the small heat shock proteins in regulating vascular smooth muscle tone. J. Am. Coll. Surg. 201, 30−36. https://doi.org/10.1016/j.jamcollsurg.2005.03.017.
Middleton, D., Williams, F., Meenagh, A., Daar, A.S., Gorodezky, C., Hammond, M., Nascimento, E., Briceno, I., Perez, M.P., 2000. Analysis of the distribution of HLA-A alleles in populations from five continents. Hum. Immunol. 61, 1048. https://doi.org/10.1016/S0198-8859(00)00178-6.
Miller, F.N., Joshua, I.G., Anderson, G.L., 1982. Quantitation of vasodilator induced macromolecular leakage by in vivo fluorescent microscopy. Microvasc. Res. 24, 56–67. https://doi.org/10.1016/0026-2862(82)90042-5.
Nielsen, K.L., McLennan, N., Masters, M., Cowan, N.J., 1999. A single-ring mitochondrial Chaperonin (Hsp60-Hsp10) can substitute for GroEL-GroES in vivo. J. Bacteriol. 181, 5871–5875. PMC94114.
Nielsen, M., Lundegaard, C., Worning, P., Lauemøller, S.L., Lamberth, K., Buus, S., Brunak, S., Lund, O., 2003. Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. Protein Science : A Publication of the Protein Society 12, 1007–1017. https://doi.org/10.1110/ps.0239403.
Nobuoka, D., Yoshikawa, T., Takahashi, M., Iwama, T., Horie, K., Shimomura, M., Suzuki, S., Sakemura, N., Nakatsugawa, M., Sadamori, H., Yagi, T., Fujiwara, T., Nakatsura, T., 2013. Intratumoral peptide injection enhances tumor cell antigenicity recognized by cytotoxic T lymphocytes: a potential option for improvement in antigen-specific cancer immunotherapy. Cancer ImmunolImmunother 62, 639–652. https://doi.org/10.1007/s00262-012-1366-6.
Owen, J., Punt, J., Stranford, S., Pat, J., 2013. Immunology, Seventh ed. W.H. Freeman and Company, New York.
Parham, P., 2005. MHC class I molecules and KIRs in human history, health and survival. Nat. Rev. Immunol. 5, 201–214. https://doi.org/10.1038/nri1570.
Pekiner, F.N., Aytugar, E., Demirel, G.Y., Borahan, M.O., 2013. HLA-A, B (Class I) and HLA-DR, DQ (Class II) antigens in Turkish patients with recurrent Aphthous ulceration and Behcet's disease. Med PrincPract 22, 464–468. https://doi.org/10.1159/000348366.
Potocnakova, L., Bhide, M., Pulzova, L.B., 2016. An introduction to B-cell epitope mapping and in Silico epitope prediction. J Immunol Res 2016, 6760830. https://doi.org/10.1155/2016/6760830.
Rehermann, B., 2016. Peptide-dependent HLA-KIR-mediated regulation of NK cell function. J. Hepatol. 65, 237–239. https://doi.org/10.1016/j.jhep.2016.05.008.
Rubinstein, N.D., Mayrose, I., Halperin, D., Yekutieli, D., Gershoni, J.M., Pupko, T., 2008. Computational characterization of B-cell epitopes. MolImmunol 45, 3477–3489. https://doi.org/10.1016/j.molimm.2007.10.016.
Rubinstein, N.D., Mayrose, I., Martz, E., Pupko, T., 2009. Epitopia: a web-server for predicting B-cell epitopes. BMC Bioinform. 10, 287. https://doi.org/10.1186/1471-2105-10-287.
Salerno-Gonçalves, R., Sztein, M.B., 2006. Cell-mediated immunity and the challenges for vaccine development. TrendsMicrobiol 14, 536–542. https://doi.org/10.1016/j.tim.2006.10.004.
Samandary, S., Kridane-Miledi, H., Sandoval, J.S., Choudhury, Z., Langa-Vives, F., Spencer, D., Chentoufi, A.A., Lemonnier, F.A., BenMohamed, L., 2014. Associations of HLA-A, HLA-B and HLA-C alleles Frequency with prevalence of herpes simplex virus infections and diseases across global populations: implication for the development of an universal $CD8^+$ T-cell epitope-based vaccine. Hum. Immunol. 75, 715–729. https://doi.org/10.1016/j.humimm.2014.04.016.
Sela-Culang, I., Kunik, V., Ofran, Y., 2013. The structural basis of antibody-antigen recognition. Front. Immunol. 4, 302. https://doi.org/10.3389/fimmu.2013.00302.
Shevtsov, M., Huile, G., Multhoff, G., 2017. Membrane heat shock protein 70: a theranostic target for cancer therapy. Phil. Trans. R. Soc. B. 373, 20160526. https://doi.org/10.1098/rstb.2016.0526.
Shiina, T., Hosomichi, K., Inoko, H., Kulski, J.K., 2009. The HLA genomic loci map: expression, interaction, diversity and disease. J. Hum. Genet. 54, 15–39. https://doi.org/10.1038/jhg.2008.5.
Sidney, J., Southwood, S., Mann, D.L., Fernandez-Vina, M.A., Newman, M.J., 2001. Sette A: majority of peptides binding HLA-A*0201 with high affinity crossreact with other A2-supertype molecules. Hum. Immunol. 62, 1200. https://doi.org/10.1016/S0198-8859(01)00319-6.
Sorger, P.K., Pelham, H.R.B., 1988. Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. Cell 54, 855–864. https://doi.org/10.1016/S0092-8674(88)91219-6.
Stangl, S., Gehrmann, M., Riegger, J., Kuhs, K., Riederer, I., Sievert, W., Hube, K., Mocikat, R., Dressel, R., Kremmer, E., Pockley, A., Friedrich, L., Vigh, L., Skerra, A., Multhoff, G., 2011. Targeting membrane heat-shock protein 70 (Hsp70) on tumors by cmHsp70.1 antibody. Proc. Natl. Acad. Sci. U. S. A. 108, 733–738. https://doi.org/10.1073/pnas.1016065108.
Subbarao, A., Kalmár, É., Csermely, P., Shen, Y., 2004. Hsp90 isoforms: functions, expression and clinical importance. FEBS Lett. 562, 11–15. https://doi.org/10.1016/S0014-5793(04)00229-7.
Takiguchi, M., Matsuda, T., Tomiyama, H., Miwa, K., 2000. Analysis of three HLA-A*3303 binding peptide anchors using an HLA-A*3303 stabilization assay. Tissue Antigens 55, 296–302. https://doi.org/10.1034/j.1399-0039.2000.550402.x.
Tenzer, S., Peters, B., Bulik, S., Schoor, O., Lemmel, C., Schatz, M.M., Kloetzel, P.M., Rammensee, H.G., Schild, H., Holzhütter, H.G., 2005. Modeling the MHC class I

pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class I binding. Cell. Mol. Life Sci. 62, 1025–1037. https://doi.org/10.1007/s00018-005-4528-2.

Tilly, K., McKittrick, N., Zylicz, M., Georgopoulos, C., 1983. The dnaK protein modulates the heat-shock response of Escherichia coli. Cell 34, 641–646. https://doi.org/10.1016/0092-8674(83)90396-3.

Tomar, N., De, R.K., 2010. Immunoinformatics: an integrated scenario. Immunology 131, 153–168. https://doi.org/10.1111/j.1365-2567.2010.03330.x.

Van Regenmortel, M.H.V., 1996. Mapping epitope structure and activity: from one-dimensional prediction to four-dimensional description of antigenic specificity. Methods 9, 465–472. https://doi.org/10.1006/meth.1996.0054.

Wang, P., Sidney, J., Dow, C., Mothé, B., Sette, A., Peters, B., 2008. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. PLoSComput Biol. 4, e1000048. https://doi.org/10.1371/journal.pcbi.1000048.

Wisniewska, M., Karlberg, T., Lehtiö, L., Johansson, I., Kotenyova, T., Moche, M., Schüler, H., 2010. Crystal structures of the ATPase domains of four human Hsp70 isoforms: HSPA1L/Hsp70-hom, HSPA2/Hsp70-2, HSPA6/Hsp70B', and HSPA5/BiP/GRP78. PLoS One 5, e8625. https://doi.org/10.1371/journal.pone.0008625.

Yamada, N., Ishikawa, Y., Dumrese, T., Tokunaga, K., Juji, T., Nagatani, T., Miwa, K., Rammensee, H.G., Takiguchi, M., 1999. Role of anchor residues in peptide binding to three HLA-A26 molecules. Tissue Antigens 54, 325–332. https://doi.org/10.1034/j.1399-0039.1999.540401.x.

Zhou, G., Levitsky, H., 2012. Towards curative cancer immunotherapy: overcoming posttherapy tumor escape. ClinDevImmunol, 124187. https://doi.org/10.1155/2012/124187.