International Journal of Advanced Technology in Engineering and Science
Vol. No.4, Issue No. 05, May 2016
www.ijates.com
ijates
ISSN 2348 - 7550

SVM BASED PREDICTION OF MAJOR HISTOCOMPATIBILITY COMPLEX BINDERS: EXPRESSION AND ANALYSIS OF *Mesobuthus Tamulus* PEPTIDE

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ABSTRACT

Mesobuthus tamulus is an Indian red scorpion, which is the most lethal species of the Buthidae family in India. *In this research work, we predict the peptide binders of neurotoxin from* Mesobuthus tamulus*sequence to MHC-I molecules are as 11mer_H2_Db, 10mer_H2_Db, 9mer_H2_Db, 8mer_H2_Db.Also study integrates prediction of peptide MHC class I binding; proteasomal C terminal cleavage and TAP transport efficiency by using sequence and properties of the amino acids. We also found the binding of peptides to different alleles by using Position Specific Scoring Matrix. Neurotoxin from* Mesobuthus tamulus*is64 residues long with 56 nonamers having antigenic MHC binding peptides. PSSM based server will predict the peptide binders of neurotoxin from* Mesobuthus tamulus*sequence to MHCII molecules are as I_Ab.p, I_Ad.p, I_Ag7, which are found antigenic epitopes region in neurotoxin from* Mesobuthus.

Keywords: neurotoxin, Peptide, Antigen, MHC, TAP, PSSM

I. INTRODUCTION

Mesobuthus tamulus is widespread across vegetated lowlands with subtropical to tropical, humid climate, lives close to human settlements, especially in rural areas. Mesobuthus tamulus, an Indian red scorpion, is the most lethal species of the Buthidae family in India. Mesobuthus tamulus venoms consist of a several toxins that are associated with high morbility and mortality, especially among the childrens. Accidents caused by scorpion are a relatively common event in tropical and subtropical countries. The effect and symptoms of the sting start immediately with a few minutes after the sting and usually progress to a maximum severity within 5 hours, i.e., vomiting, profuse salivation, sweating, nausea, irritability, hyperthermia, and convulsion. Clinical signs and symptoms observed in humans and experimental animals are related with an excessive systemic host inflammatory response to stings and stings, respectively [1-5]. However, Identification of antigenic peptide that binds to MHC [Major Histocompatibility Complex]- molecule improves the understanding of specificity of immune responses and is important for discovery of vaccines. MHC molecules are cell surface proteins that found in all vertebrate animals and are play an important role in the immune system.

1.1 MHC Class I Antigen

In the Antigen-presentation process, antigenic protein (neurotoxin from *Mesobuthus tamulus)* is cleaved into oligopeptides in the proteasome [4-6], then cleaved fragments of peptides enter the endoplasmic reticulum (ER) via TAP protein where these peptides form a complex with MHC class I molecule [7-11]. Then these complexes are translocated towards the surface of the antigen presenting cell, where they are recognized by T-cell to elicit an immune response [12-16]. Therefore, prediction of TAP binding peptides is important for identification of the MHC class-1 restricted T cell epitopes.

1.2 Proteasomal Degradation

Proteasomal degradation is important step in the antigen-presentation process to regulate the balance between intracellular proteins [17]. Inside the proteasome by the action of proteinase the antigenic protein (neurotoxin from *Mesobuthus tamulus)* are cleavaged into oligopeptides [18] and then these oligopeptides are binds to the TAP, which transports these peptides into the ER.

1.3 Transport of peptide by TAP

TAP is heterodimeric transmembrane protein, is a family of ABC transporter that transports antigenic peptide (neurotoxin from *Mesobuthus tamulus)* into ER [19] because most of the MHC binding peptides are unable to diffuse across membrane, but TAP able to carry them inside the ER where it binds to MHC class I molecules. These MHC-peptide complexes will be translocate on the surface of antigen presenting cells [20] and are recognized by T-cell receptors to elicit an immune response.

1.4 MHC Class II Antigen

In the extracellular antigen presentation, prediction of peptides binding to a MHC class II molecule is difficult due to different side chains and longer length [21-23]. In the MHC class II antigen presentation process, antigenic protein neurotoxin from *Mesobuthus tamulus*are ingested by antigen-presenting cells through the process of endocytosis or phagocytosis, then cleaved by cathepsins a class of protease into oligopeptides in the endosomes, than are fuse with lysosomes containing MHC class II molecules [24] and present them at the cell surface for recognition by T cells [25-33]. Where T helper cells trigger an immune response by inflammation and swelling due to phagocytes or may lead to an antibody-mediated immune response via B-cell activation. Since MHCs have a key role in immune system by stimulating cellular and humoral immunity against neurotoxin from *Mesobuthus tamulus*and are used for controlling specific immunological processes by creating peptides to bind to specific MHC alleles and this binding affinity to specific peptides are used for designing synthetic peptide vaccines [34-37].

II. MATERIALS AND METHODS

2.1 Predictions of MHC class I binding peptide

MHC binding peptide is predicted using neural networks trained on C terminals of known epitopes. By using RANKPEP [34] we predict peptide binders to MHCI molecules from protein sequences or sequence alignments

using Position Specific Scoring Matrices (PSSMs) whose C terminal end is likely to be the result of proteosomal cleavage.

2.2 Prediction of Antigenic Peptides by Cascade SVM based TAPPred method

By using TAPPred we predict TAP binders on the basis of sequence and the properties of amino acids. We found the MHCI binding regions [Table-3], the binding affinity of neurotoxin from *Mesobuthus tamulus* having 64 amino acids, which shows 56nonamers.

2.3 Predictions of MHC class II binding peptide

MHC peptide binding of neurotoxin from *Mesobuthus tamulus*is predicted using neural networks trained on C terminals of known epitopes. By using RANKPEP we predict peptide binders to MHCII molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). MHC molecule binds to some of the peptide fragments generated after proteolytic cleavage of antigen.

III. RESULTS AND INTERPRETATION

In this research work, we predict the peptide binders of neurotoxin from *Mesobuthus tamulus*sequence to MHC-I molecules are as 11mer_H2_Db, 10mer_H2_Db, 9mer_H2_Db, 8mer_H2_Db [Table-1]. MHC molecule binds to peptide fragments which are generated after proteolytic cleavage of antigen tend to be high-efficiency binders. TAP is an important transporter that involved in the translocation of peptides from cytosol to ER. TAP binds and translocates selective peptides for binding to specific MHC molecules. Therefore, predicting binding affinity of those peptides toward the TAP transporter is crucial to identify the MHC class-1 restricted T cell epitopes. Cascade based support vector machine shows 15 High affinity TAP binder residues at N and C termini using sequence and properties of the amino acids of neurotoxin from *Mesobuthus tamulus*[Table-3]. This method integrates prediction of peptide MHC class I binding; proteasomal C terminal cleavage and TAP transport efficiency by using sequence and properties of the amino acids. We also found the binding of peptides to different alleles by using Position Specific Scoring Matrix. Neurotoxin from *Mesobuthus tamulusis*64 residues long with 56nonamers having antigenic MHC binding peptides. PSSM based server will predict the peptide binders of neurotoxin from *Mesobuthus tamulus*sequence to MHCII molecules are as I_Ab.p, I_Ad.p,I_Ag7 which are found antigenic epitopes region in neurotoxin from *Mesobuthus tamulus*[Table-2].

Table -1 Peptide binders of neurotoxin from *Mesobuthus tamulus*to MHC-I molecules, having C-terminal ends are proteosomal cleavage sites.

MHC-I Allele	POS	N	SEQUENCE	C	MW (Da)	SCORE	% OPT
8mer_H2_Db	7	GYI	ADGDNCTY	ICT	839.83	19.409	36.97 %
8mer_H2_Db	8	YIA	DGDNCTYI	CTF	881.91	16.284	31.02 %
8mer_H2_Db	18	ICT	FNNYCHAL	CTD	963.08	2.657	5.06 %
8mer_H2_Db	55	PTP	VPIRGSGK	CR	794.95	2.514	4.79 %
8mer_H2_Db	11	DGD	NCTYICTF	NNY	946.1	0.624	1.19 %

9mer_H2_Db	16	TYI	CTFNNYCHA	LCT	1054.16	23.475	46.61 %
9mer_H2_Db	7	GYI	ADGDNCTYI	CTF	952.99	21.29	42.27 %
9mer_H2_Db	22	NNY	CHALCTDKK	GDS	1000.19	4.452	8.84 %
9mer_H2_Db	39	CDW	WVPYGVVCW	CED	1044.3	1.977	3.93 %
9mer_H2_Db	43	VPY	GVVCWCEDL	PTP	982.17	1.602	3.18 %
9mer_H2_Db	21	FNN	YCHALCTDK	KGD	1035.2	0.592	1.18 %
10mer_H2_Db	16	TYI	CTFNNYCHAL	CTD	1167.32	10.739	18.25 %
10mer_H2_Db	6	DGY	IADGDNCTYI	CTF	1066.15	2.075	3.53 %
11mer_H2_Db	15	CTY	ICTFNNYCHAL	CTD	1280.48	19.833	24.95 %

* The RANKPEP consists of a list of selected peptides binding potential (score) to the MHC molecule from the query given at a selected threshold. Peptides shown here contain a C-terminal residue that is predicted to be the result of proteasomal cleavage and also focus on the prediction of conserved epitopes that help to avoid immune evasion resulting from mutation. Proteasomal cleavage options are only applied to the prediction of MHCI-restricted peptides.

Table -2 *Cascade SVM based High affinity TAP Binders of* neurotoxin from *Mesobuthus tamulus*

Peptide Rank	Start Position	Sequence	Score	Predicted Affinity
1	31	GDSGACDWW	8.608	High
2	32	DSGACDWWV	8.325	High
3	21	YCHALCTDK	8.242	High
4	40	VPYGVVCWC	8.013	High
5	33	SGACDWWVP	7.781	High
6	43	GVVCWCEDL	7.636	High
7	15	ICTFNNYCH	7.591	High
8	41	PYGVVCWCE	7.452	High
9	47	WCEDLPTPV	7.281	High
10	38	WWVPYGVVC	7.183	High
11	29	KKGDSGACD	6.844	High
12	24	ALCTDKKGD	6.788	High
13	14	YICTFNNYC	6.774	High
14	36	CDWWVPYGV	6.633	High
15	5	YIADGDNCT	6.421	High

* TAPPred showing Cascade SVM based High affinity TAP Binders sites, their sequence, rank, position and scores are displayed in the tabular output are to be found 15 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini neurotoxin from *Mesobuthus tamulus*.

Table - 3 *Peptide binders of* neurotoxin from *Mesobuthus tamulusto MHC-II molecules*.

MHC-II Allele	POS	N	SEQUENCE	C	MW (Da)	SCORE	% OPT
I_Ab	21	FNN	YCHALCTDK	KGD	1035.2	11.9	33.40 %
I_Ad	19	CTF	NNYCHALCT	DKK	1020.14	11.561	21.75 %
I_Ad	22	NNY	CHALCTDKK	GDS	1000.19	9.676	18.21 %
I_Ad	12	GDN	CTYICTFNN	YCH	1060.2	7.231	13.61 %
I_Ag7	13	DNC	TYICTFNNY	CHA	1120.24	14.634	35.80 %
I_Ag7	20	TFN	NYCHALCTD	KKG	1021.13	12.35	30.22 %

IV. CONCLUSION

The Antigenic proteins (neurotoxin from *Mesobuthus tamulus*involved multiple antigenic components to direct and empower the immune system to protect the host from the neurotoxin. Major histocompatibility complexes (MHC-I and MHC-II) display specificity to bind with their respective epitopes. MHC class molecules are cell surface proteins that take active part in host immune reactions to response almost all antigens. This knowledge of the immune responses to an antigen protein (neurotoxin from *Mesobuthus tamulus)* clear that the whole protein is not necessary for raising the immune response, but a small fragment of antigen can induce immune response against whole antigen. This means the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of neurotoxin from *Mesobuthus tamulus*, hence are helpful *in silico* to design and develop highly predictive computational tools for the identification of T-cell epitopes. Finally, accurate prediction remains vital for the future to design synthetic peptide vaccine.

Abbreviations

MHC - Major Histocompatibility Complex

TAP -Transporter associated with antigen processing

PSSM- Position Specific Scoring Matrices

SVM - Support Vector Machine

Conflict on Interest - None

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