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Predicting Multi-Epitope Peptide Cancer Vaccine from Novel TAA Topo48

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Abstract: Cancer is one of the most lethal diseases. Recently, cancer immunotherapy has a tremendous achievement in cancer treatment. A certain number of cancer based epitope vaccines with different moiety have been discovered. In japan, several clinical tests of cancer based epitope vaccine derived from tumor associated antigens (TAAs) are now ongoing or have recently been completed. A novel of TAAs potentially as cancer vaccine have been retrieved from a fragment weighed 48kDa derived from human DNA-topoisomerase 1 (TOP1) called Topo48. Therefore, it is still critical to discover a derived Topo48 epitope based cancer vaccine. Immuno-informatics considered as a methods noted to have better accuracy to design promising vaccine candidates. Here, continuous and discontinuous B-cell epitopes following with CTL epitopes and their docking interaction to major histocompatibility complex (MHC) class I Human Leukocyte Antigens (HLA)- A0201 were predicted. Kolaskar-Tongaonkar's, Emini's, Karpus-Schulz's, and Parker's methods were used to predict continuous B-cell epitopes while ElliPro was used for prediction of discontinued B-cell epitopes. Those considered methods marked to have better accuracy to design promising vaccine candidates. Similarly, CTL epitopes was also predicted by using NetCTL server and the best candidates were further investigated their binding affinity by mean of PEP-FOLD3, PatchDock rigid-body docking server, and FireDock server. Total of 27 continuous epitopes and 7 discontinuous B-cell epitopes were predicted. In the other hand, 9 peptides were predicted as CTL epitopes. Whereas, three predicted CTL epitope in range 263MLDHEYTTK27, 755AIDMADEDY763, 715ALGTSKLNY724 exhibited good interactions to HLA-A0201. Moreover, we also found residues His266, Thr270, Ala755, Tyr723, Thr718, Ser719, Lys720 from Topo48 and residues Thr163, Asp757, His70, Glu63 from HLA- A0201 were indicated to be antigenic. Ultimately, our proposed continuous/discontinuous B-cell epitopes, and also CTL epitopes can be potential vaccines for cancer immunotherapy.

Keywords: *Epitopes; HLA; Immuno-informatics; MHC class 1; Topo48; TAAs*

Introduction

Cancer is the second leading cause of death globally and accounted for 8.8 million deaths in 2017. Lungs, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervix and stomach cancer are the most common among women. Interestingly, between 30 % and 50 % of cancer death could be prevented by modifying or avoiding key risk factors, including avoiding tobacco products, reducing alcohol consumption, maintaining a healthy body weight, exercising regularly and addressing infection-related risk factors; however, there are no definite treatment is available [1].

Recently, cancer immunotherapy has a tremendous achievement in cancer medications [2]. Specificity eradication and fewer serious undesirable non-specific toxicity have been strongly linked to major advantage of immunotherapy [3]. Naturally, immune systems have ability to recognize and harness the immune system power to maintain it into eliminating cancerous cells. Mostly, the cytotoxic T cells (CTL) and B cells are the major agents in the cancerous immune system. CTL has been shown a special effector not only kill the cancerous cell directly but also mounting the immune respond such as recruiting type 1 helper T cells (Th1), natural killer cells (NK), macrophage and dendritic cells to help them eliminating cancerous cells. B cell is well known as humoral immune responses, immunoglobulins (Ig)

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produced immediately after they are activated, can recognize specific marker on cancerous cells [4].

The CTL and B cell epitopes have played a critical role to exponentially mount and guide an immune system to kill cancerous cells [5]. A certain number of cancer based epitope vaccine with different moiety have been discovered [6]. Normally, the investigation of cancerous epitopes has been linked to a specific biomarker, commonly called tumor associated antigens (TAAs) [7]. In Japan, several clinical tests of cancer based epitope vaccine are now ongoing or have recently been completed [8]. They have been grouped into series of TAAs and type of cancers: E75 and GP2 epitopes from Her2 TAAs breast cancer, E7 and E6 epitopes from TAAs Wilms-tumor and human papilloma, etc. However, most of vaccines demonstrate not fully induce cellular immunity [9]. Until now, several cancer epitopes have been roughly discovered and pushed into the clinical trials, but unfortunately, only E75 and GP2 having been being reported in clinical trial [10]. Interestingly, one epitope designed from Her2 with different area to E75 and GP2 called CH401MAP could induce specific antibody to reduce breast cancer proliferation Her2 positive through high affinity interaction to class I HLA [11]. Another peptide vaccine derived from mouse and monkey model cause antibody-induce cytokine storm in healthy donor in clinical trial [12]. Therefore, it is still critical to discover a new epitope based cancer vaccine.

The advance development of bioinformatics techniques and applications along with experimental data progression has led to rapid development in the field of computational immunology (in silico). In the other hand, a number of researches in field immunology-focused resources and software will help in understanding of immune system properties [13]. Base on this stories, the immuno-informatics has been available as a new branch of bioinformatics to solve the immunological problems. By mean of immuno-informatics along with docking as well as the molecular modelling tools, the investigation and predicting potential CTL and B cell epitope as a new vaccine can be done [14].

Recent studies reveal that in silico approach helps the in vitro experiments to build a basic foundation of designing new vaccines. Indeed, the prediction of B-cell epitopes and CTL epitopes by mean of in silico approaches have led to vaccine candidates reported by researcher to produce promising preclinical and clinical trial results [15]. Among in silico methods, Kolaskar and Tongaonkar's methods was reported to have 75% experimental accuracy to predict continuous antigenic epitopes of certain sequence TAAs [16]. In the other hand, other methods such as Emini [17], Karpus and Schulz [18], and Parker methods [19] have been successfully predicted of surface accessibility, flexibility, and hydrophilicity, respectively. Those considered methods noted to have better accuracy to design promising vaccine candidates [20].

Following which, a novel of TAAs potentially as cancer vaccine have been announced from a fragment weighed 48 kDa derived from human DNA-topoisomerase 1 (TOP1) called Topo48 [21]. This TAAs seem to be potential since their shown to be associated with early stage oesophageal squamous cell carcinoma (ESCC), gastric acid (GC), colorectal cancer (CRC) and non-small-cell lung cancer (NSCLC). TOP1 is an enzyme that plays a critical role in cancer development [22]. Its function resolves the topological problem of tangled DNA during the transcription stages [21], [22]. Because of the fact that TOP1 has been reported to have over expressed in many of cancer types, the certain chemotherapeutic agent such as doxorubicin, etoposide, etc., has been designed to decrease their activities. It has been proved that the high rate TOP1 antigen in the cancer cells make them visible to immune system: therefore, its epitope could be potential to design a new epitope cancer vaccine candidate.

Here, in this study, since Top48 has been reported as a novel TAAs and it is no sufficient information of vaccines derived from Top48 and also with the help of immuno-informatics approach, we tried to make potential B-cell and CTL epitope that can be characterized as effective vaccine candidates. By mean of appropriate molecular modelling tools, peptide epitopes-MHC complexes were modelled. To further investigation, molecular docking also employed to further study selecting of the potential candidates for cancer peptide vaccines.

Method

Retrieval of Topo48 sequences

The primary Topo48 sequences were retrieved from DNA topoisomerase 1 sequence (sp|P11387) stored in the National Centre of Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/protein/P11387/). PDB ID 1A31 was used for sequence materials. The initial sequence length for Topo48 were 436 amino acids started from amino acid sequence 329 to 765 [21].

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Prediction of continuous B-cell epitopes

Antigenicity for Topo48 was predicted by using Immune Epitope Database Analysis Resource (IEDB) (http://tools.immuneepitope.org/tools/bcell/iedb_input) by Kolaskar and Tongaonkar antigenicity methods. Antigenic properties such as hydrophilic nature and accessibility characteristics for a flexible region of immunogen were investigated by using Parker hydrophilicity prediction [19], Emini prediction of surface accessibility [17], Karplus and Schulz flexibility prediction [18], respectively.

Topo48 homology modeling and structure validation

Homology modeling was performed by using SWISSMODEL [19]. By using certain Topo48 sequence, the appropriate templates of three-dimensional (3D) coordinate structures were taken from the Protein Data Bank (PDB) database by mean of SWISSMODEL server. Following which, the three dimensional structure of Topo48 was then modelled. The validation stereo-chemical quality of modelled structures was verified by PROCHECK [23].

Prediction of discontinuous B-cell epitopes

Ellipro from IEDB (http://tools.immuneepitope.org/tools/ElliPro/iedb_input) was performed for prediction of conformational (discontinuous) epitopes [24]. Protrusion Index (PI) of residues, neighboring residues clustering based on PI and protein shape approximation were employed as algorithms.

Prediction of CTL epitopes

NetCTL.1.2 server (http://www.cbs.dtu.dk/services/NetCTL) [25] were employed to predict CTL epitopes. Briefly, the FASTA sequence was performed as input. Then, human leukocyte antigen (HLA) alleles and peptide lengths were submitted. Afterwards, the predicted T-cell epitopes were as output. Here, the MHC class 1 binding, the proteasomal C-terminal cleavage and TAP transport efficiency were predicted.

Molecular docking studies of CTL epitopes-HLA-A0201

The predicted CTL epitopes that contained antigenic amino acids were selected. Molecular modeling 3D structures of these peptide antigens were performed by using PEP-FOLD3 server [26] by using 300 simulation runs. Different conformational models base on sOPEP energy appeared as the results. The best ranked models were docked to the selected MHC class I molecule namely, HLA-A (PDB ID:2GIT) [27]. The docking was done by using PatchDock rigid-body docking server [28]. The docking results were then refined and scored with FireDock server [29]. Finally, interaction properties such as hydrogen bonding interaction were analyzed with UCSF Chimera 1.12 [30].

Results And Discussion

Immunotherapy including multi peptide vaccination is the major considered as the most effective method to prevent cancer diseases [2]–[4]. The foundation of peptide vaccines is based on identification of B-cell and Cytotoxic T-cell (CTL) epitopes which are immuno-dominant and inducible to specific immune responses to foreign substances or malignances [14]. The rapid growth of bioinformatics techniques has addressed to new field called immuno-informatics. It is branch of bioinformatics which deal with in silico analysis and modelling based immunological data [31]. By mean of immuno-informatics, several limitations with respect to the conventional experimental methods in vitro and/or in vivo, prediction and analysis of the complete spectrum of potential antigens is possible. Following which, time and cost consuming addressed to conventional experiment also can be reduced by this method [32]. Moreover, vaccines designed by using in silico approaches have been tested over to clinical trials [33].

A novel TAAs Topo48 has been recently reported as promising target to cure kind of cancers such as, early stage oesophageal squamous cell carcinoma (ESCC), gastric acid (GC), colorectal cancer (CRC) and non-small-cell lung cancer (NSCLC) [21]. Until now, there are no vaccine derived from TAAs; therefore, its epitope could be potential to design a new epitope cancer vaccine candidate. Moreover, beside of its immunogenicity, other hallmark such as expression level, oncogenicity role and several antigen epitopes are the kind of consideration to make it as potential antigen for cancer vaccines [21], [22].

An effective cancer vaccine must include B-cell continuous (linear) or discontinuous (conformational) epitopes in regard to induce T-helper (CD4+) and T-cytotoxic (CD8/CTL) make sure cellular and humoral system are running up [14]. In addition, the suitable cancer vaccine should consist of CTL epitopes as helper and adjuvant part [34]. Therefore, in this study, B-cell epitopes and CTL epitopes have been predicted by using immuno-informatics approach.

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Four methods have been used to predict appropriate continuous antigenic epitopes of Topo48. Firstly, the 27 antigenic epitopes in range 6 – 47 amino acids were retrieved from Kolaskar and Tongaonkar method's some of them also predicted as CTL epitopes and residue cysteine (C) at position 504th found in the antigenic peptide position 501 to 518 (501TVGCCSLRVEHINLH PEL518) was observed to have the highest residual score of 1.179 (**Table 1**).

Table 1. Predicted Antigenic B-Cell epitope from the Top48 protein

No	Start position	End position	Peptides	Peptide length
1	112	117	SPPQIK	6
2	124	130	GYFVPPK	7
3	183	188	KKVPEP	6
4	217	263	GKVMKLSPKAEEVATF**FAKM**	47
5	294	303	ITNLSKCDFT	10
6	305	311	MSQYFKA	7
7	335	344	LKEYGFCIMD	10
8	352	360	NFKIEPPGL	9
9	380	398	EDIIINCSKDAKVPSPPPG	19
10	410	417	VTWLVS**WT**	8
11	423	430	SIKYIMLN	8
12	450	457	LKK**CVDKI**	8
13	473	488	RQRAVALYFIDKLALR	16
14	501	518	TVG**C**CSLRVEHINLHPEL	18
15	521	530	QEYVVEFD**FL**	10
16	535	546	IRYYNKVPVEKR	12
17	548	554	FKNLQLF	7
18	573	581	LNKHLQDLM	9
19	584	591	**LTAKVFRT**	7
20	593	606	NASITLQQQLKELT	14
21	613	619	**PAKILSY**	7
22	624	635	RAVAILCNHQRA	12
23	667	673	SAKADAK	7
24	680	695	TKKVVESKKKAVQRLE	16
25	697	706	QLMKLEVQAT	10
26	713	719	QI**ALGTS**	7
27	721	743	LNYLDPRITVAWCKKWGVPIEKI	23

Note: Residues (in underline) were predicted to have residual score greater than 1.000; Residues (in bold) were also predicted as CTL epitopes; Residue (in green bold) was predicted to have the maximum residual score

Expectedly, residue Cys504 (**C**) will be play a critical role in continuous B-cell epitope. This method was employed due to well reported and appreciated to give 75 % experimental accuracy [16]. Secondly, the surface accessibility and flexibility were performed by Emini et al. [17], and Karplus and Schulz's scale flexibility method [18]. Residue glutamic acid (E) at position 160th from antigenic hexapeptides (158KKEKKR163) predicted to have surface probability score 6.998. it means that E160 is the surface residue within > 20 Å distance to water (**Figure S1a**). Whereas, according to Karplus and Schulz's scale flexibility method [18], residue Serine (S) at position 73th from antigenic heptapeptide

(70KDGSSEK76) was found to have the highest maximum flexibility score (1.127) (**Figure S1b**). The higher the maximum flexibility score, the more flexible structure it is [19]. This method calculates B factor or temperature to indicate atomic vibration/motion within the structure. Then, the Surface hydrophilic region was predicted by Parker hydrophilicity scale method [20]. Residue aspartic acid (D) at position 143th from pentapeptide position 140 to 146 (140RDEDDAD146) was found to have the maximum hydrophilicity score 7.729 (**Figure S1b**). This method was useful to help the researcher purifying peptide based on the peptide retention times during HPLC. Moreover, surface hydrophilic sites are well known as antigenic site in the immunological studies [20]. Overall, the predicted epitopes, surface accessibility, flexibility and hydrophilicity could be helpful in cancerous continuous B-cell epitopes development.

Despite the fact that B-cell epitopes are continuous [35], it has been reported that approximately > 90 % of B-cell epitopes are discontinuous [36]. Therefore, predicting discontinuous Topo48 B-cell epitopes also urgently need to investigate. For the first of all, sufficient 3D conformational structure was done by using SWISSMODEL. Server found four 3D structure that have 90 % identity (**Table 2**).

Table 2. Homology template parameter for Topo48

Template PDB ID (chain)	Query cover	E-Value	Confidence	Maximum identity
1A31 (A)	77 %	0.0	89 %	99 %
1K4S (A)	77 %	0.0	89 %	99 %
1SC7 (A)	77 %	0.0	90 %	99 %
1K4T (A)	77 %	0.0	89 %	99 %

The percentage identity between sequence and template in range > 30-40 % indicate extremely high accurate to model. While the confidence value was found to be > 89 % overall indicates that the model has high accuracy within 2-4 Å rmsd from native structure. Structure validation PROCHECK analysis also performed to verify the stereo-chemically and geometrically. Ramachandran plot quality (**Figure 1**) reveals that modelled 3D structure of Topo48 is good condition.

After verified Topo48 3D structure is retrieved, ElliPro is then employed to predict its discontinuous epitopes.

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ElliPro is an extraordinary accurate tool based webserver to predict discontinuous epitopes [24].
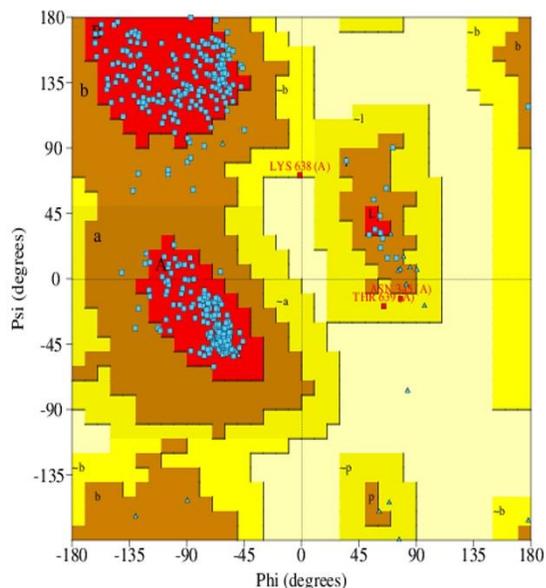

Figure 1. Ramachandran plot of modelled Topo48 3D structure

This tools correlates among solvent accessibility, antigenicity and flexibility of protein, structurally. In the other hand, it also capable to differentiate epitopes based on protein-antibody interactions. Seven discontinuous B-cell epitopes in range 3 – 20 and 30 - 44 amino acids were collected within protrusion index (PI) value above 0.7 and the highest PI score is 0.931 calculated to peptide comprised by 44 amino acids (**Figure 2**). PI value represent the percentage of protein atom that extend out of molecule bulk (ellipsoid) and are involved in antibody binding [37].

In regard to predict CTL epitopes, those epitopes must be good binder to MHC class I. Further interaction between T-cell receptor (TCR) from CTL and peptide (epitope) bound MHC class I is critical step to stimulate CTL response [37]. The processes of peptide bound MHC is established by various steps. First, TAAs over expressed in cancer will be high rate ubiquitous, which are processed by proteasome into oligopeptides, usually 8-10 amino acids, the peptides enter into endoplasmic reticulum (ER), peptides are bound on MHC class I via transporter associated with antigen presenting (TAP) then the complex peptide-MHC class I is presented on the cancerous cells [37].

Here, CTL epitopes were predicted by Net CTL server. Basically, this tool is a method that integrates the

prediction of peptide MHC class I binding and TAP efficiency. Nine nonapeptides predicted whose prediction score epitopes > 0.75 were retrieve from Topo48 protein. Six of them also demonstrated as B-cell linear epitope (**Table 3**).
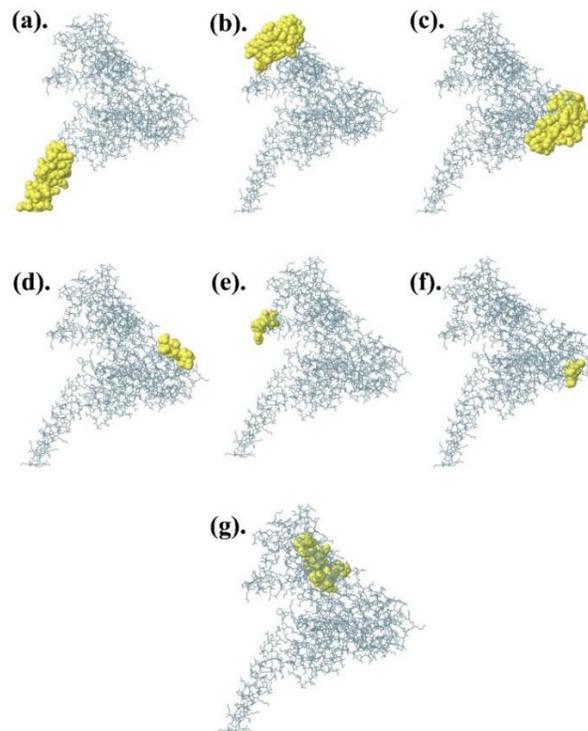

Figure 2. 3D Representation of the predicted discontinuous epitopes. Yellow color indicates the region of discontinuous epitopes. Backbone licorice represent the conformational 3D structure of Topo48.

Table 3. Predicted CTL epitope from Topo48 protein

Residue number	Peptide sequence	MHC binding affinity	C-terminal cleavage affinity	Prediction score	MHC ligand
260	**FAKML**DHEY	0.2345	0.6415	1.2304	YES
263	**M**LDHEYTTK	0.1619	0.8726	0.8411	YES
416	**W**TENIQGSI	0.2285	0.2153	1.0221	YES
453	**CVDKI**RNQY	0.5281	0.9726	2.5353	YES
529	**FL**GKDSIRY	0.2285	0.8743	1.2285	YES
584	**LTAKVFRT**Y	0.5453	0.9525	2.6027	YES
611	NIP**AKILSY**	0.1696	0.9758	1.0119	YES
715	**ALGTS**KLNY	0.2221	0.8377	1.2152	YES
755	AIDMADEDY	0.5184	0.937	2.49	YES

Note: Prediction score threshold was set at >0.75000; Residues (black bold) indicates that amino acid also predicted as antigenic B-cell sites; Residues (red bold underline) predicted to have residual score greater than 1.000, also antigenic B-cell sites.

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In addition, their modelled 3D structures generated by PEP-FOLD are displayed in **Figure 3**. Mostly, six CTL epitopes have helix shapes and others have turn shapes. Their contained residues are responsible for their different shapes.
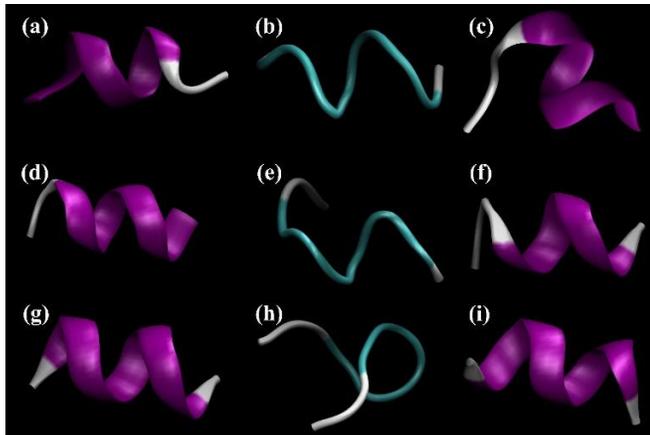

Figure 3. Predicted CTL epitopes generated by EliPro and modelled by PEP-FOLD. (a) 260FAKMLDHEY268, (b) 263MLDHEYTTK272, (c) 416WTENIQGSI425, (d) 453CVDKIRNQY461, (e) 529FLGKDSIRY537, (f) 548LTAKVFRTY592, (g) 611NIPAKILSY620, (h) 715ALGTSKLNY724, (i) 755AIDMADEDY763.

To investigate those CTL epitopes interactions with MHC class I protein especially on HLA-A*02:01, molecular docking was performed by PatchDock [28] then FireDock [29] assist in resolving flexibility scoring (refinement). Prior to employ docking studies, nine 3D structure of predicted CTL epitopes were modelled with PEP-FOLD3 [26]. Finally, docking study reveals that out of nine epitopes, three CTL predicted epitope (263MLDHEYTTK27, 755AIDMADEDY763, 715ALGTSKL NY724) exhibited good interactions to HLA-A0201 (**Figure 3b, i, h**). Post-docking analysis (**Table S1**) results also revealed that in 263MLDHEYTTK27-MHC HLA-A0201 complex (**Figure 4b**), eight hydrogen bonds were found within a filtered distance of 3.5 Å, and residues His266, Thr270 from peptide and Thr163 associated in hydrogen bond were predicted as antigenic. Peptide 755AIDMADED Y763, were comprised by six hydrogen bond within distance of 3.5 Å, where the residue Ala755 from the peptide epitope and Asp757 significantly involve in hydrogen bond were expected as antigenic. On the other hand, the third peptide (715ALGTSKLNY724) contained five hydrogen bond, and significant residues Tyr723, Thr718, Ser719, Lys720 from peptide and His70, Glu63 involved in hydrogen bond were also predicted as antigenic. All molecular interactions of the nine predicted CTL epitopes following with their poses are demonstrated in **Figure 4**.
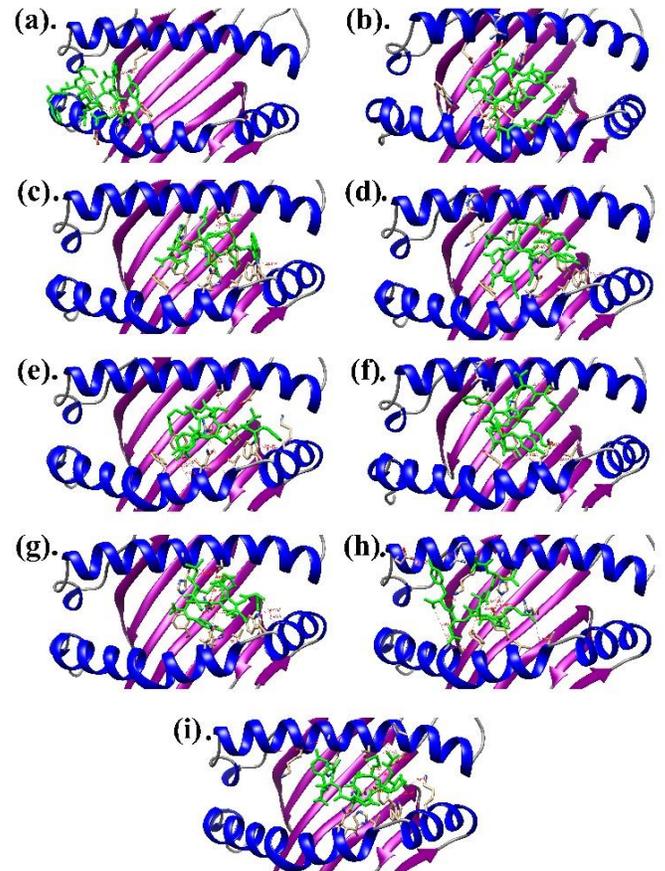

Figure 4. Molecular interaction analysis of predicted peptide derived Topo48 (green) docked to MHC-1 HLA A*02:01 (blue-purple); a) 260FAKMLDHEY268, (b) 263MLDHEYTTK272, (c) 416WTENIQGSI425, (d) 453CVDKIRNQY461, (e) 529FLGKDSIRY537, (f) 548LTAKVFRTY592, (g) 611NIPAKILSY620, (h) 715ALGTSKLNY724, (i) 755AIDMADEDY763.

Conclusions

B-cell and T-cell epitopes prediction has major implementation in cancerous vaccine development. Immune system can be guide to react with the certain generated antigenic epitopes. Several tools have been used to observe the hallmark features of novel TAAs Topo48 protein including antigenicity, surface accessibility, flexibility, and hydrophilicity of continuous B-cell epitopes, discontinuous B-cell epitopes. In addition, CTL epitopes and their interaction properties are also predicted and analyzed. Ultimately, through our immuno-informatics study, several novel Topo48 epitopes were generated. We also inferred that those predicted epitopes display therapeutic potential to further investigation in vitro and/or in vivo. Moreover, further used for preclinical and clinical in cancer vaccine development.

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Conflicts of interest

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