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An *In silico* Chimeric Vaccine Targeting Breast Cancer Containing Inherent Adjuvant

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Background: Today, Lack of efficient therapeutic strategy for breast cancer (the most common cause of death in women) is one of the momentous problematic topics for all health care committees. Designing new specific vaccine, based on antigens located on the surface of cancer cells can be useful. Over expression of ROR1, lacked of HER2/neu, and hormone receptors on cell surface in the breast cancer, introduce this protein as an appropriate candidate for designing cancer vaccine.
Objectives: We hypothesized the extracellular domain of receptor tyrosine kinase like orphan receptor 1 (ROR-1) along with a super antigen such as staphylococcal enterotoxin B could be a potent vaccine for drug resistant breast cancer.
Materials and Methods: Here, we assessed the findings of bioinformatics analysis to identify the antitumor immune properties of this chimeric construct. In addition, the stability, physic-chemical properties and allergic potency of designed fusion protein were investigated by valid bioinformatics software.
Results: Our result suggested that chimeric model is capable to be a stimulant of both T-cell and B- cell mediated immune responses with an acceptable accessibility and solubility but without any allergenicity.
Conclusions: The ROR-1 with an enterotoxin B could be a potent vaccine for breast cancer.

Keywords: ROR-1; Staphylococcus Entorotoxin B; Breast Neoplasms; Vaccines

1. Background

Breast cancer is one of the most common causes of death in women all around the world. It is estimated that more than 1.38 million new women suffered from breast cancer (1). Anti-estrogen therapy is the choice strategy for estrogen receptor positive breast cancer patients. However, a large number of breast cancers do not respond to this type of treatment because of lack of estrogen receptor. Furthermore, another group of breast cancer becomes insensitive to hormone therapy after first therapy and immediately promotes to grow (2). Although chemotherapy is an alternative strategy for treating insensitive and metastatic breast cancer, but many of the treated cancers often develop a recurrence. Therefore, innovating novel agents is an urgent requirement for attenuating the mortality rate (3). The receptor tyrosine kinase like orphan receptor 1 (ROR1) is a transmembrane protein and belongs to the receptor tyrosine kinase family (4). This protein has five domains, three extracellular including an immunoglobulin like motif, frizzled and Kringle domains, transmemebrane part and an intracellular tyrosine kinase domain (5). Over expression of ROR1 is found in the embryonic stage (6) and several cancers including B-CLL (7), B-ALL (8), gastric carcinoma, non-small cell carcinoma cell lines (9) and breast cancer (10). Zhang et al. revealed the overexpression of ROR1 in the breast cancer related with lacked of HER2/neu and hormone receptors. Moreover, upregulation of ROR-1 in primary breast cancer is associated with poor differentiation and shorter survival rate (10). There are limited or lack of ROR-1 in normal cells while over expression of this protein has been reported among human cancer cells, therefore ROR1 may be applied as a potent target for immunotherapy.

Staphylococcal enterotoxin B (SEB), a 28 KDa superantigen, is a powerful T cell activator. This protein binds to MHC class II on antigen presenting cells (APCs) and then forms complex with the variable region of β chain of T cell receptor. The binding site of SEB on the APCs has differed from that of specific antigens (11). SEB exerts a potent mitogenic effect on both CD4+ and CD8+, increasing cytokines including interferon-γ (INF-γ), interleukin 2 (IL-2), and tumor necrosis factor-α (TNF-α), finally promotes a powerful antitumor immunity (12).

One of the main goals of immunotherapy against tumor is to create a specific tumoral antigen response that participates to the tumor eradication. Designing the combined construct, enabling to activate both cellular

and humoral anti-tumor immunity is an effective therapeutic method which restricts or eradicates tumor progression. To date, it is possible to design a suitable combined construct, based on B cell and the T cell epitope map using bioinformatics methods.

2. Objectives

Here, we purposed to design the immunotherapeutic target constructed having two parts. The first one was extracellular domains of ROR1 as specific tumoral antigens for inducing B cell lymphocyte and second one is SEB, an adjuvant to create anti-tumoral response via T cell lymphocyte.

3. Materials and Methods

3.1. Protein Sequences and Designing the Construct

To obtain the protein sequences of ROR-1 and SEB, the UniProtKB database was used. The accession number of SEB and ROR-1 was P01552 and A2VCQ3, respectively. To make a fusion protein based on ROR-1 and SEB having robust specific anti-tumoral activity, the extracellular part of ROR-1 containing the frizzled domain and Ig-like C2 type domain was selected and joined to the complete sequence of SEB with GSGGSGGSGGSG as a hydrophobic amino acid linker. To survey the antigenicity of designed construct, the online database VaxiJen v2.6 was utilized.

3.2. The Physico-Chemical Characteristics

The physiochemical features, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average hydropathy of recombinant construct were analyzed by the Expasy ProtParam server (http://us.expasy.org/tools/protparam.html).

3.3. Prediction of Secondary Structure

To assess the secondary structure of ROR-1-SEB fusion protein, the online database GOR IV (13) were performed. To confirm findings from Gor4, fusion proteins evaluated by PHDsec (https://www.predictprotein.org).

3.4. Prediction of 3D structure

The 3D model of the designed construct, was evaluated by the I-TASSER online server (14). To compute the Energy minimization and Ramachandran plot of the suggested 3D model, Swiss-PdbViewer and Procheck server (15) were executed.

3.5. Prediction of B-Cell and T-Cell Epitopes

To predict the linear and conformational B-cell epitopes, full-length primary sequences of designed fusion protein were computed using BCPreds (16) and web server CBTOPE (17), respectively. In addition, for prediction of discontinuous B-cell epitopes from three-dimensional protein structures, Discotope server was employed (18). Furthermore, to identify the antigenicity of selected BCPreds epitopes with the cutoff value of > 0.8, the VaxiJen (threshold = 0.4, ACC output) was used. On the other hand, BCPreds software was utilized for predicting continuous B cell epitopes based on different parameters including hydrophilicity, plasticity, exterior accessibility, antigenicity, flexibility, surface exposed, and polarity along full length designed construct. To survey MHC Class I and MHC Class II binding common epitopes, Propred-1 (47 MHC Class I alleles) (19) and Propred (51 MHC Class II alleles) (20) servers was exerted, respectively. In accordance with two mentioned servers, the whole numbers of MHC allele interaction were estimated. The antigenicity value of predicted epitopes was analyzed by VaxiJen.

3.6. Prediction of Allergenic Regions

To recognize the possibility of existence of allergenic regions, AlgPred was used. The server predict allergens along the fusion protein sequence in accordance with similarity to known epitopes. Next the allergenicity was evaluated through SDAP database (21).

3.7. Prediction of Protein Solubility

To assess the protein solubility, the recombinant protein solubility prediction was exerted (22).

4. Results

4.1. Designing the Construct

The extracellular part of ROR1 was selected for designing the N-terminal of chimeric because of its accessibility to antigen presenting cells. This part has consisted of the Frizzled domain, which involves in proliferation, cell polarity and cell developing, and Ig-like C2 type domain, which participates in cell-cell recognition and immune system stimulation. Full- length of SEB was conjugated with GSGGSGGSGGSG linker to ROR1 and formed C-terminal of the described construct. Figure 1 depicts the schematic diagram of chimeric construct using DOG 1.0 software (23). Based on VaxiJen outcomes, the antigenicity index of ROR1 fragment alone, SEB fragment, linker and the combination of all three mentioned parts were 0.6488, 0.5618, 4.9849 and 0.5994.

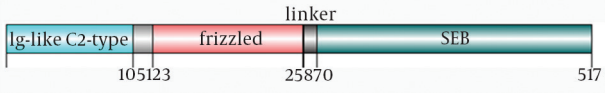


Figure 1. Schematic representation of ROR1-SEB constructs containing two extracellular domains of ROR1 (Ig-like C2 type and Frizzled domain) and the whole sequence of SEB fusing together with a hydrophobic linker

4.2. The Physico-Chemical Characteristics

Our construct has 517 amino acids consisting 65 negatively charged residues and 62 positively charged residues. Its molecular weight was 59.0199 KDa. The value of pI was 6.42 that indicated the acidity feature of the designed construct. The extinction coefficient of ROR1-SEB was 58,065 M-1 cm-1 at 280 nm. The appraised half-life was > 10. In accordance with an instability index of ExPASy, ProtParam (< 40), ROR1-SEB was categorized as an unstable protein (instability index=41.90). The alphabetic index and Grand average of hydropathicity of ROR1-SEB was 70.48 and -0.527, respectively.

4.3. Prediction of Secondary Structure

Figure 2 illustrates the pattern of secondary structure of ROR1-SEB predicted by GOR IV. Based on our findings, the structural content of ROR1-SEB was composed of 18.64% alpha helix, 27.38% extended strands and 53.98% random coil. Full length of ROR1-SEB is made up of 33 random coils, 30 extended strands and 11 alpha helices. As summarized in Table 1, the secondary structure pattern of the chimeric protein is similar to the extracellular part of ROR1 and SEB.

4.4. Prediction of 3D Structure

As findings from I-TASSER server, five three-dimensional models were afforded for our designed protein fusing with GSGGSGGSGGSG as a linker. Figure 3 illustrates the best tertiary model predicted for describing protein, which has three separate parts and two domains. The confidence score, as a factor estimates the quality of suggested model was -2.22. What's more, the expected TM- score and RMSD were 10.9 ± 4.6 Å and 0.53 ± 0.15, respectively. Additionally, we fused two proteins using (HDPVRVS) 2 as an alternative construct. I-TASSER results from predicting tertiary models for this alternative construct showed no appropriate structure therefore we exerted all our analysis only on the first construct. The Ramachandran plot assessment viewed that 80.2% (413 amino acids), 11.1% (57 amino acids) and 8.7% (45 amino acids) were situated in the favored region, allowed region and outlier region, respectively (Figure 4). The quality assessment of the Ramachandran plot revealed that more than 90% of residues located on acceptable (favored and allowed) regions. In accordance with Pdb Viewer analysis, the energy minimization amount was -9536.065 Kcal/mol that portended the plausible stability for our designed construct.

4.5. B-Cell Epitopes

Table 2 listed the epitopes predicted by BCpreds and AAPpreds within the full-length of designing protein. The appropriate epitopes were selected according to cutoff values of 0.8, 0.8 and 0.4 for BCpreds, AAPpreds and VaxiJen, respectively. Moreover, the conformational B cell epitopes were evaluated by two servers termed Discotope and CBTOPE and their data summarized in Tables 3 and 4, respectively. On the other hand, the predicted B cell epitopes were determined in accordance with different parameters including hydrophilicity, flexibility, accessibility, exposed surface, polarity and antigenic propensity with the respective thresholds of 1.9, 2, 1.9, 2.4, 2.3, 1.8 and 1.9, respectively. As demonstrated in Table 5, although applied linker has been determined as a hydrophobe and flexible epitope, it showed no surface exposed epitope, having to interact with antibodies. Table 6 summarizes the predicted epitopes, which can simultaneously interact with B cell, MHC class I and class II with the highest number.

4.6. Allergenicity Property

Based on outcome from AlgPred and SDAP database, our construct viewed no allergenic sites along its sequence. Furthermore, it had no great similarity to the allergen listed in SDAP library.

4.7. Protein Solubility Prediction

According to outputs from recombinant protein solubility prediction, our designed construct possesses 36.6% a solubility chance after overexpressin in *E.coli*.

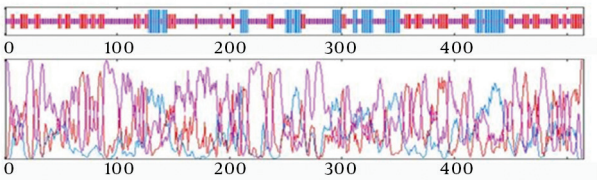


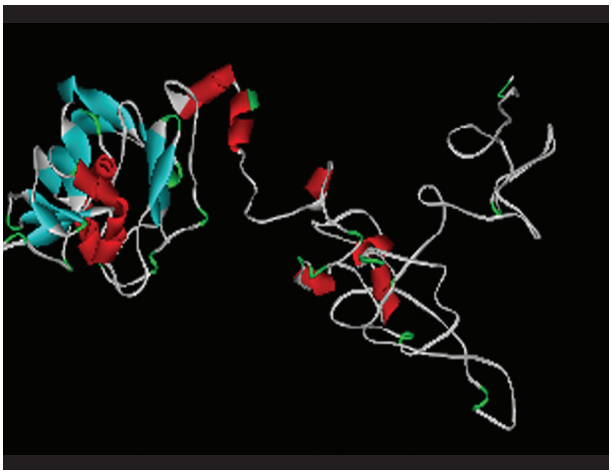
Figure 2. Graphical outcomes for predicting the secondary structure of ROR1-SEB. Blue, purple and red indicate the Helix, extended strand and random coiled structures, respectively

Table 1. Pattern of Secondary Structure of Origin and Chimeric Proteins [a]

Protein	Extended Strand	Alpha Helix	Random Coil
ROR1 (extracellular part)	26.78	9.62	63.60
SEB	28.95	25.19	45.86
ROR1-SEB	27.38	18.64	53.98

[a] **Data are presented as %.**

Figure 3. A Probabilistic Structural Model for Chimeric Protein Using I-TASSER Software



As illustrate in figure two domains of Ror1 (in the left site) separate with the linker from SEB fragment (in the right site).

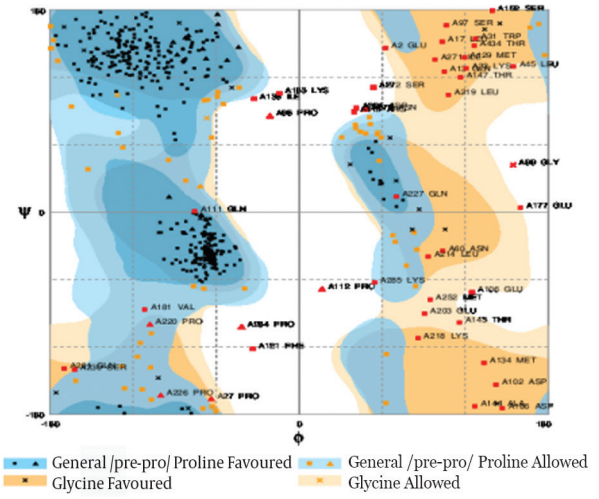


Figure 4. A Feature of Ramchandran Plot for the ROR1-SEB Chimeric Protein

Table 2. B-Cell Epitopes Mapping by BCPred Results (BCPred + AAP)

Position	BCpred Epitope	Score	VaxiJen	Position	AAPpred Epitope	Score	VaxiJen
89	KFGPPPTASPGYSDEYEEDG	1	0.1212	279	ESQPDPKPDELHKSSKFTGL	1	0.9262
240	GSGGSGGSGGSGMYKRLFIS	1	0.8888	34	NDAPVVQEPRRLSFRSTIYG	1	1.0983
498	NKMVDSKDVKIEVYLTTKKK	1	0.7183	494	MYNDNKMVDSKDVKIEVYLT	1	0.3562
23	GNPPPTIRWFKNDAPVVQEP	0.999	0.8779	88	VKFGPPPTASPGYSDEYEED	1	0.0531
278	AESQPDPKPDELHKSSKFTG	0.968	0.7827	468	IENENSFWYDMMPAPGDKFD	1	0.6351
216	RLKLPNCEDLPQPESPEAAN	0.915	0.7060	226	PQPESPEAANCIRIGSGGSG	1	1.1506
1	DEPMNNITTSLGQTAELHCK	0.881	0.8571	12	GQTAELHCKVSGNPPPTIRW	1	1.0859
470	NENSFWYDMMPAPGDKFDQS	0.868	0.5261	392	MYGGVTEHNGNQLDKYRSIT	1	1.4149
167	LCHYAFPYCDETSSVPKPRD	0.867	0.6159	351	LADKYKDKYVDVFGANYYYQ	0.869	0.3303
353	DKYKDKYVDVFGANYYYQCY	0.805	0.3624	62	DTTDTGYFQCVATNGKEVVS	0.092	0.6347
374	SKKTNDINSHQTDKRKTCMY	0.788	0.2889				
63	TTDTGYFQCVATNGKEVVSS	0.779	0.6283				
331	IKDTKLGNYDNVRVEFKNKD	0.74	1.0925				

Table 3. Findings From Discotope Server for Conformational B-Cell Epitope Mapping

Amino Acid	Position	Contact Number	Discotop Score
DEPMNN	1- 6	1,16, 7, 19, 14, 6	0.515, -0.842, 0.634, -1.893, -1.431, -1.81
TTSLGQT	8-14	9,15, 4, 0,2, 8, 3	-2.375, -3.332, -1.213, -0.741, -0.858, -2.744, -2.486
ELHCKVSGNPPPTIRW	16-31	12, 22, 14, 21, 6, 20, 2, 14, 2, 4, 0, 8, 10, 16, 16, 15	-3.248, -3.584, -2.197, -2.664, 0.005, -1.423, 1.683, 1.138, 2.601, 2.668, 3.306, 2.322, 1.075, -0.466, -1.82, -3.433
EPRRLS	41-46	3, 0, 2, 9, 16, 2	-3.138, -1.218, -0.279, -0.751, -2.35, -0.849
RST	48-50	2, 15, 1	-1.325, -3.416, -2.109
GS	53-54	5, 2	-2.365, -3.229
DT	65-66	0, 3	-2.461, -2.745
GP	91-92	11, 3	-3.196, -2.418
PT	94-95	12, 3	-2.906, -2.069
SP	97-98	0, 3	-0.66, -2.636
QPESP	227-231	3, 10. 3, 7, 1	-2.599, -2.678, -2.72, -1.993, -2.921
GSGGSGGSGGSGM	240-252	7, 13, 8, 11, 3, 9, 10, 4, 3, 5, 6, 8, 12	-0.897, -1.514, 0.086, 1.325, 3.232, 1.298, 0.689, 1.171, 0.916, -0.147, -1.799, -3.045, -3.294
QPDPKPDELH KSSKFTGLME N	281-301	18, 12. 9, 21, 5, 8, 20, 7, 26, 26, 9, 28, 8, 12, 31, 2, 15, 7, 23, 4, 23	-3.327, -2.155, -1.736, 0.317, 4.474, 3.591, 3.562, 5.622, 1.649, 2.357, 5.92, 2.29, 5.097, 4.013, 1.748, 4.057, 2.026, 2.515, 0.336, 1.913, -1.357
KV	303-304	20, 13	-3.060, -2.894
DD	307-308	13, 6	-3.677, -3.135
INVKSIDQF	314-322	5, 6, 24, 6, 34, 25, 21, 16, 21	0.162, 0.84, -2.32, 1.272, -2.386, -2.22, -1.957, -1.63, -3.409
Y	324	5	-2.481
I	328	18	-2.524
S	330	13	-0.9
KDTKLGNYDN	332-341	4, 24, 3, 7, 14, 2, 14, 23, 10, 24	-0.679, -3.185, -0.899, -1.087, -1.69, 0.423, -1.37, -2.883, -1.184, -2.874
NKD	348-350	6, 1, 3	-2.495, -1.55, 0.181
ADK	352-354	20, 3, 17	-3.396, -0.961, -1.437
KDKYV D	356-361	15, 2, 21, 7, 31, 18	-1.009, 2.356, -0.582, 0.301, -3.061, -2.35
YQ	369-370	14, 13	-2.388, -2.661
Y	372	1	-1.604
SKKTNDINSHQTDKRK	374-389	5, 23, 14, 17, 4, 0, 11, 2, 0, 2, 14, 2, 3, 27, 13, 24	-0.966, -2.147, 0.302, 0.523, 2, 4.992, 2.571, 5.378, 5.726, 5.674, 1.902, 3.401, 3.032, -1.421, -1.065, -3.076
TEHNGNQLDKYRS	397-409	30, 10, 18, 6, 4, 25, 10, 16, 0, 5, 10, 23, 7	-3.34, 0.052, 0.2, 2.671, 2.901, -0.518, 1.283, 0.41, 2.285, 0.737, -0.385, -2.808, -2.969
EDGK	416-419	15, 7, 1, 4	-2.338, 1.239, -0.095, -3.019
Q	427	8	-3.343
NKKK	429-432	14, 26, 9, 16	0.718, -1.554, 1.49, 1.2
N	449	20	-3.366
K	451	13	-1.034
YEFNNSPY ETGY	453-464	27, 13, 0, 2, 9, 38, 10, 29, 12, 16, 31, 23	-1.439, 0.886, 4.002, 4.59, 2.755, -0.539, 2.565, -0.529, 3.315, 1.136, -1.455, -1.643
YD	476-477	28, 21	-3.446, 1.018
MPAPGDKFD	479-487	30, 30, 10, 11, 5, 0, 6, 29, 10	0.017, 1.22, 4.186, 4.109, 3.864, 4.003, 2.213, -0.442, 0.496
K	490	9	-2.104
ND	496-497	17, 9	-3.3, -2.01
K	499	18	-2.508
D	502	9	-2.631
K	504	8	-3.009
YLTTKKK	511-517	17, 27,16, 8, 12, 8, 8	-3.5, -3.402, 0.009, 1.758, 3.135, 3.925, 2.506

Table 4. Conformational B-Cell Epitopes Predicting by CBTOPE Server

Amino acid	Position	Probability Scale	Amino Acid	Position	Probability Scale	Amino Acid	Position	Probability Scale
DEPM	1-4	4	M	129	5	VFGAN	362-366	4
HC	18-19	4	ESLH	130-133	4	YYQCYFSKKTNDI	368-380	4
V	21	4	M	134	5	S	382	4
S	22	6	QG	135-136	4	QT	384-385	4
GN	23-24	5	NQITAA	140-145	4	D	386	5
P	25	4	M	148	4	K	387	7
P	26	5	T	151	4	R	388	5
P	27	4	S	152	5	K	389	6
T	28	5	SH	153-154	4	TC	390-391	5
IRWFKN	29-34	4	C	159	4	M	392	4
PVVQEPRRLS	37-46	4	PSLCHYAFPYC	165-175	4	YGGVTEH	393-398	5
I	51	4	DETS	176-179	5	NGNQLDKY	400-407	6
RLRI	55-58	4	SVPKPRDLCRDE	180-191	4	R	408	7
NLDT	60-63	4	C	192	5	S	409	6
T	66	4	EILENVLCQTEYI	193-205	5	IT	410-411	5
G	67	5	PESPEAANCI	228-237	4	V	412	4
YF	68-69	4	V	276	4	G	418	4
VAT	72-74	4	AESQPDPKPDEL	278-289	4	SFDV	423-426	4
GK	76-77	4	H	290	5	LV	446-447	4
SST	81-83	4	KSS	291-293	4	K	448	5
PTA	94-96	4	MENMK	299-303	4	NKKLYEFN	449-456	4
SDEYEEDGF	101-109	4	LY	305-306	4	DQSKYLMMY	487-495	4
C	110	5	D	308	4	D	497	4
QP	111-112	4	H	310	4	KMVDSKD	499-505	4
ARFI	119-122	4	TKLGNY	334-339	4	KI	507-508	4
RT	125-126	4	NV	341-342	4	Y	511	4
V	127	5	VEFKN	344-348	4			
Y	128	4	DL	350-351	4			

Table 5. Findings From Bcepred Software for B-Cell Epitope Based on Discrepant Parameters

Prediction Parameters	Epitope Position
Hydrophilicity	59 - 68, 97 - 108, 135 - 141, 174 - 182, 226 - 235, 240 - 252, 278 - 288, 307 - 313, 347 - 359, 374 - 391, 397 - 406, 425 - 423, 456 - 463, 483 - 490, 496 - 503
Flexibility	153 - 159, 172 - 181, 237 - 250, 277 - 283, 287 - 295, 362 - 370, 372 - 388, 423 - 431, 481 - 487, 499 - 505, 510 - 517
Accessibility	1 - 7, 22 - 49, 54 - 68, 89 - 109, 124 - 130, 135 - 141, 177 - 190, 217 - 235, 278 - 297, 303 - 312, 329 - 361, 363 - 392, 396 - 410, 412 - 418, 423 - 494, 500 - 517
Exposed surface	40 - 46, 101 - 107, 179 - 185, 279 - 294, 329 - 335, 342 - 361, 372 - 391, 401 - 409, 425 - 437, 445 - 456, 484 - 491, 499 - 505, 511 - 517
Polarity	14 - 22, 38 - 49, 53 - 61, 100 - 109, 133 - 139, 182 - 196, 254 - 266, 282 - 296, 340 - 361, 382 - 393, 413 - 419, 426 - 437, 442 - 456, 465 - 472, 484 - 490, 499 - 517
Antigenic propensity	16 - 23, 37 - 43, 66 - 72, 74 - 92, 164 - 170, 191 - 204, 255 - 265, 267 - 276, 319 - 331, 357 - 364, 366 - 375, 409 - 416, 427 - 430, 440 - 448, 505 - 514

Table 6. The List of Epitopes Having Both the B- and T-Cell Immune Responses Throughout the Length of the ROR1-SEB Chimeric Protein

Sequence	Number of Mhc Class I Binding Alleles	Number of MHC Class II Binding Alleles	Vaxijen Scores	Total Number of MHC Binding Alleles
PMNNITTSL	3	30	0.5520	33
VEFKNKD	14	26	-0.2150	40
ATNGKEVV	4	5	0.6692	9
ESPEAANCI	14	0	0.6486	14
NGKEVVss	5	6	-0.2295	11

5. Discussion

Development of efficacious therapeutic strategy for some of the resistant malignancies is the main emergency of health organization all around the world. Nowadays, design of appropriate and safe vaccines which stimulate the immune response actively or passively, are the hot topic in the field of reverse vaccinology. This area is closely related to computational vaccinology that recruits discrepant informatics tools to predict efficient T- and B- cell functional epitopes to improve the properties of an antigen based vaccine (24, 25).

The principal purpose of the current study was to design a unique construct, including two antigenic parts, which adjoined together through hydrophobic linker (26, 27). Theoretically, our structural model could augment immunogenicity of ROR-1 protein and owing to the presence of staphylococcal enterotoxin B as a potent superantigen; its probability evokes a wide cellular or humoral anti-tumor immune response. As respects to the momentous role of linker in representing the pattern of various epitopes throughout the chimeric protein besides maintenance of its functional properties, the linker selection is a key point in designing of the fusion protein (26). In this study a flexible linker, GSGGSGGSGGSG With 12aa, was used to separate domains of two proteins. Aria et al. repotted the multimerizing property of short helical linker compared with longer ones. Furthermore, a flexible linker based on shorter conformation plays an efficient role in comparison to those with the helical linker (26). To predict the secondary structure of a chimeric protein, the GOR method was applied. This software allows estimating the possible secondary structure of each amino acid together with its impact on the condition and structure of adjacent amino acids. The most abundant structure within our fusion protein was a random coil that could be due to the presence of a high amount of hydrophobic amino acids such as glycin. In accordance with finding from the physico-chemical parameter analysis, our fusion protein had an acidic nature with the high extinction coefficient at 280 nm, which is owing to high content of Cys, Trp and Tyr. In contrast to partial instability of our fusion protein, its estimated high alphabetic index infers to protein stability in a broad range of temperature.

One of the most important problems in the designing of recombinant protein is the biologic functional characteristics. Although prediction of secondary structure by ab-initio methods or folding recognition is able to detect some of the limitations (28), prediction of three-dimensional structure through comparative and ab-initio methods attenuates several errors (29, 30). The three-dimensional model of the fusion protein ROR-1-SEB protein was accounted using the I-TASSER server (14) according to their confidence score (C-score), Z-score, RMSD and TM-score. This server suggests five models for our chimeric sequence that model 2 had more c-score between them, therefore it selected for further evaluation. Expected TM-score obtained 0.53 ± 0.15, which accredited the validity of the model. A TM-score more than 0.5 portend accuracy of topology. Data from Procheck Ramachandran plot demonstrated the stability of the fusion protein. Thereabout, 8.7% of the residues located in outline region, which, could presumably be owing to fusion. Since the purpose of designing a vaccine is the generation and selection of a candidate with the potential stimulation of strong responses (31), we analyzed the epitope maps for B cell and T cells. At first, we predicted linear B-cell epitope overall the chimeric sequence using BCpreds. Moreover, for recognizing the epitopes involved in antibody-antigen interaction, the estimation of conformational epitopes is an essential in the computational vaccine design which executed using both structure and sequence information based method, including DiscoTope and CBTOPE, respectively. Our result showed the copious b-cell epitopes, though some of the solely predicted by one method. In order to predict the map of T-cell epitope and binding affinity to both classes of MHC molecules, Propred and Propred-1 were applied. Numorous T-cell epitope with a high antigenicity score were suggested by two methods, but we only selected those epitope that is simultaneously proposing as B-cell and T-cell epitope (Table 6). Our result suggested that our structural model represented the epitope that are capable to be a stimulant for both T-cell and B- cell mediated immune responses. At last, this structure showed no significant resemblance with the allergen in the SDAP library.

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Conflict of Interest

The authors made no disclosures.

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