Central Asian Journal of Medical and Pharmaceutical Sciences Innovation **2**(1): 15-24 (2022)

RESEARCH PAPER

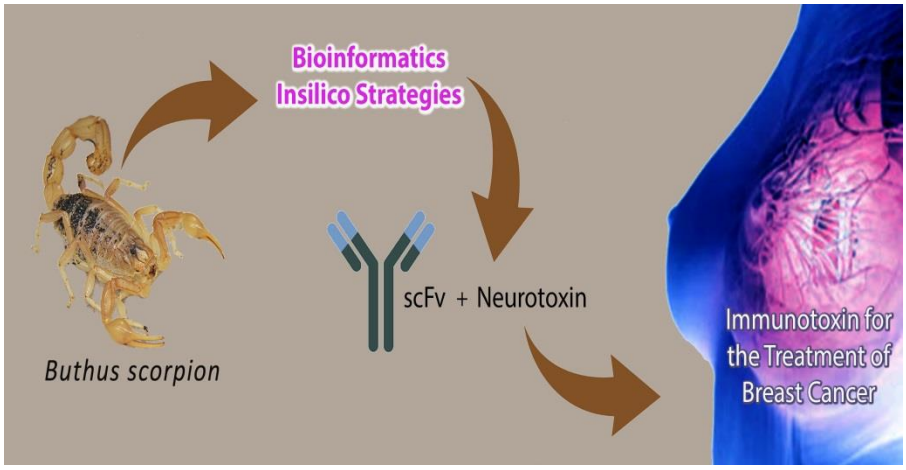
Development of a new immunotoxin from part of the scorpion venom for the treatment of breast cancer

Saeed Pirmoradi
Razi Vaccine and Serum Research Institute, Agricultural Research and Training Organization (AREEO), Ahvaz, Iran

Highlights

- Breast cancer is one of the most cancers worldwide.
- New methods of cancer treatment with the help of modern technologies in the field of software are expanding today.
- Immunotoxins based on new toxins is one of the new and effective methods in the treatment process of many common cancers in the world.
- Nowadays, drug design with the help of software is very efficient in reducing the cost and time in the field of producing new drugs.

Graphical Abstract



Article Info

Receive Date: 22 December 2021
Revise Date: 27 January 2022
Accept Date: 28 January 2022
Available online: 08 February 2022

Keywords:
Breast cancer
Neurotoxin
Immunotoxin
scFv

Abstract

Breast cancer is one of the most cancers worldwide. It is associated with many problems due to the structural heterogeneity of the treatment process. Many patients with breast cancer (HER2) are positive. Current cancer treatment methods are not very effective in reducing mortality. However, immunotherapy is less effective in treating cancer and damages the body. An anti-cancer immunotoxin molecule that contais an immune component, which is an antibody or a binding component of an antibody, and the other part is a toxin, which is a lethal compound. The anti-HER2 receptor trastuzumab is derived from a single-stranded variable fragment (scFv) that binds to part of a scorpion toxin called neurotoxin Bmk. We investigated the physicochemical properties, secondary structure, and solubility of the chimeric protein using ProtParam and GORIV, PORCalc, PepCalc, and protein-sol, respectively. The structure and solubility of the model were evaluated using PROCHECK, protein-sol, and PepCalc. ALLERTOP server was used to predict sensitivity, and mRNA stability was assessed using RNAfold. Finally, docking of immunotoxin and HER2 was performed using ClusPro server. The results showed that the chimeric protein is a protein with a stable secondary structure in solution and a three-dimensional structure, which also has a stable mRNA and can bind to HER2. The results showed a stable and soluble protein with the desired binding ability to HER2, which made it a suitable immunotoxin candidate for the treatment of breast cancer whose safety needs to be evaluated in clinical phases.

10.22034/CAJMPSI.2022.01.03 **E-ISSN: 2783-0993**

*Corresponding author: pirmoradi150@gmail.com (S. Pirmoradi)

Introduction

Breast cancer is one of the most common cancers known worldwide. Due to structural heterogeneity, the treatment process is associated with many problems (1). Therefore, modern methods of cancer treatment such as surgery, radiation therapy and chemotherapy are not effective enough in reducing mortality. Evidence shows that 25-30% of cases of human HER2 breast cancer are positive (2). HER2 or ERBB2 belongs to a group of proteins involved in signal transduction pathways and is a member of the human epidermal growth factor receptor family with intracellular domains and tyrosine kinase activity. These receptors are associated with morphogenesis, the natural regulation of cell growth, and cell differentiation (3). The significant role of HER2 in the development of cancer and its biological properties such as increased recurrence of malignancy, high risk of metastasis, resistance to conventional chemotherapy, has made it a very attractive target for cancer treatment (4). HER2 monoclonal antibody (mAb), (Herceptin) significantly improved the outcomes of HER2-positive cancers. But the development of resistance in cancer patients has limited the ability to use these agents (5). Hence, there is a need for new and advanced therapies to target HER2.

Immunotoxins (IT) are a group of targeted therapies composed of two functional proteins, mAb and toxin, which are covalently linked together. The toxin component in immunotoxins was obtained through various sources, including plant or bacterial derivatives and the venom of various organisms (6). In recent years, researchers have made great efforts to engineer living bacterial cells to target tumors and induce cell death in tumors (7). Many researchers today have also found that some scorpions, such as Buthus martensii Karsch (BmK), known as the Chinese red scorpion, belong to the Buthidea family and have many medical uses, including in the field of cancer (5, 6). The medical use of the BmK scorpion dates back to the Song Dynasty of China. To date, several active molecules have been isolated and identified from the scorpion venom as anti-epileptic, analgesic, anti-rheumatic and anti-cancer (7, 8).

Therefore, the toxic compounds in scorpion venom also have the potential to participate in the production of immunotoxins. Binding, a toxin to mAb and following HER2 dimerization, tyrosine residual phosphorylation leads to the onset of various intracellular signaling cascades such as PI3K/AKT and ERK/MAPK (aka Ras/Raf/MEK /ERK) pathways that proliferate with cell proliferation. Apoptosis is associated with initiating cell death, either by entering the cytosol and catalytically inactivating cell stem processes or by altering the membrane properties of tumor cells, allowing cells whose antigens are detected by mAbs to be selected (9, 10). Bioinformatics is a branch of science that uses various methods to solve biological problems, especially at the molecular level (11). In bioinformatics studies, related methods for DNA/protein sequence analysis and secondary prediction and tertiary structures of protein molecules, protein-protein interactions, etc. on these two chimeric proteins are studied and used (12).

In the present study, bioinformatics-based Insilico strategies for designing chimeric structures consisting of scFv and part of the venomous neurotoxin toxin derived from Buthus scorpion (Bmk) that binds to HER2-specific scFv via a flexible binder. HER2-positive breast cancer cells were used to target. Once a 3D model has been developed for this chimeric protein, its structure, stability, solubility, and binding to HER2 will be predicted and then evaluated using in silico methods.

Materials and Methods

Sequence analysis and chimeric structure design

Neurotoxin BmK and trastuzumab scFv amino acid sequences, including VL and VH sequence levels, were obtained from the NCBI database with access number(UniProt: G4V3T9.1) and the protein database (PDB ID: 1n8z), respectively. First, the VL and VH of Herceptin were ligated by the KKKKSKKKKSKSKKKKS binder to form scFv. Subsequently, the GGSGG sequence was selected as the optimal linker between BmK and scFv. In this construct, a Furin protein identification site with RGRR amino acid sequence between GGSGG linker and BmK neurotoxin fragment was also designed (Figure 1).

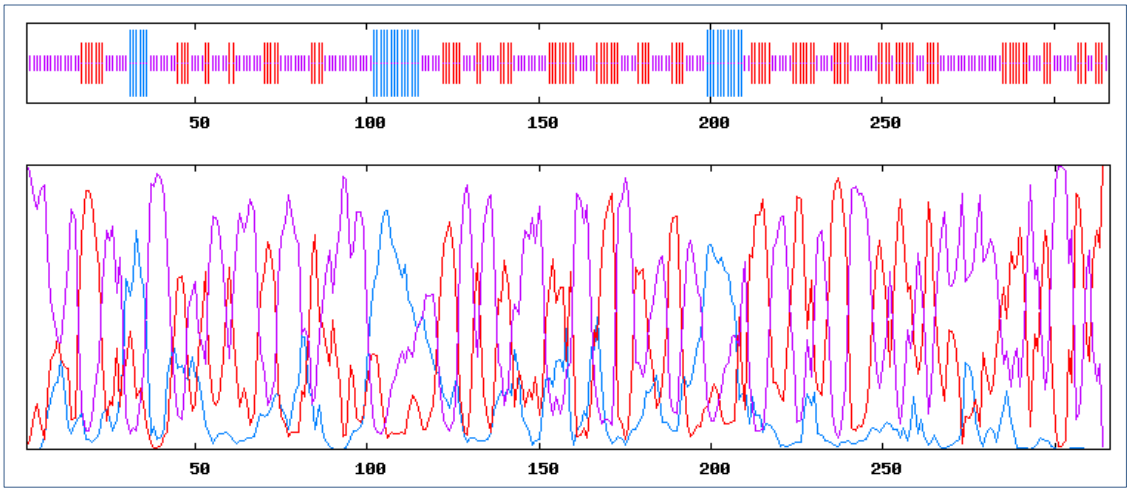


Figure 1. GORV web server results.

Predicting secondary structure

After scFv formation, using the GORV server, the secondary structure of scFv after binding to the BmK neurotoxin was analyzed via a short circuit, and finally, the percentage of secondary structures in the final structure was calculated. Physicochemical properties and predictability of solubility Various physicochemical properties of immunotoxins, including molecular weight, theoretical isoelectric point (pI), net charge, instability index, aliphatic index and large hydropathic mean (GRAVY) were analyzed using the ProtParam web server. Online protein-sol and pepCalc software were used to predict the solubility of the immunotoxin, which uses experimental data to assess the accuracy of the prediction and solubility.

Predicting, refining and validating the third structure

I-TASSER was used to investigate three-dimensional models for the chimer structure. This hybrid operating system was used to analyze protein structure and predict performance. Then PROCHECK servers were used to validate 3D models. The PROCHECK server, which evaluates the stereochemical properties of the protein structure, showed the number of residues in the desired and desirable regions and the quality of the 3D model before and after modification. Also, ALLERTOP is a bioinformatics tool used to predict the potential allergenicity of the chimeric structure.

Predicting mRNA stability

Foldweb server RNA was used to predict the RNA structure of the chimeric protein concerning the lowest energy content.

Docking immunotoxin and HER2

Docking between chimeric protein and HER2 receptor was performed using a ClusPro server. This server can estimate protein-ligand binding. HER2 and the three-dimensional structure of the scFv + Bmk chimeric protein were sent to the ClusPro server as receptors and ligands in the form of PDB. Default parameters were used to perform the calculations.

Results

Results of secondary structure prediction

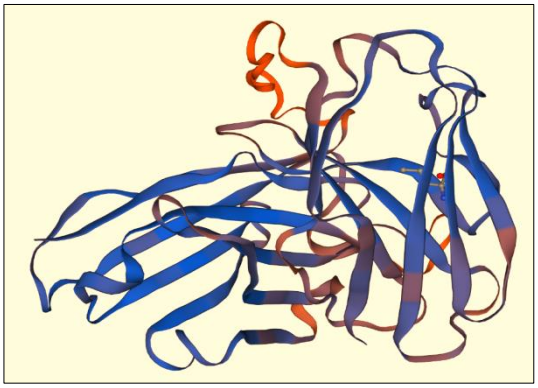
The GORV web server results showed that the connection of VL and VH through the flexible linker and constituent scFv does not change their secondary structure. In addition, the secondary structure of scFv does not change with the binding of Bmk and scFv via short linkers and the formation of scFv + Bmk. The secondary structure of the chimeric protein contains 9.81% alpha-helix, 34.18% extended strand, and 56.01% random screw (Figure 1).

Results of physicochemical properties and predictability of solubility

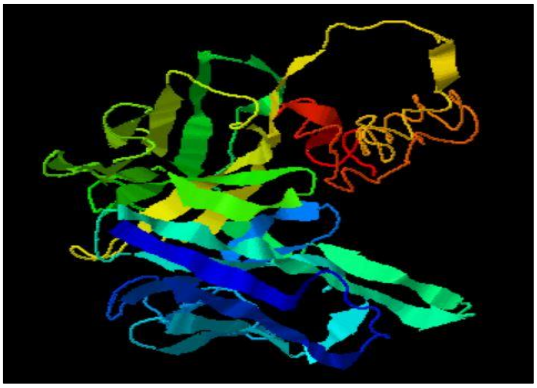
Physicochemical properties and solubility of chimeric protein sequences were observed using ProtParam and protein-sol, respectively. According to ProtParam results, molecular weight, theoretical pI and net charge were 34668.95, 9.17, and +14, respectively. The aliphatic and gravitational indices of Chimer protein were 58.32 and -0.559, respectively. scFv + Bmk was identified as a stable protein (instability index=36.58, the index below 40 means protein stability), and based on the prediction of solubility based on protein-sol server and pepCalc chimeric protein, it was shown that is a soluble protein (0.536=protein-sol solubility score) because a combination with a score above 0.45 is considered soluble protein on this server.

Results of forecasting, refining and validating the third structure

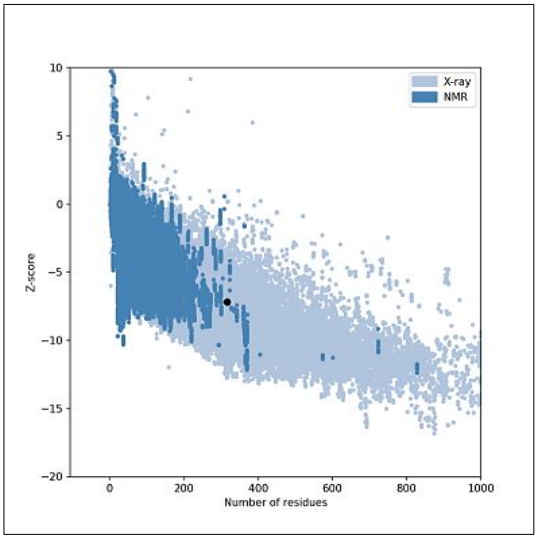
Five three-dimensional models of chimeric protein were made by I-TASSER and SWISSMODEL servers and the model with the highest reliability score (-1.94) (c- score) was selected. C scores were between -2.64 and -1.94. A higher value of the score indicates a safer model and vice versa. Among the five models obtained from the I-TASSER server, the model with the highest desirable area (64.4%) and it's allowable (26.2%) in total (90.8%) and (c-Score=-1.94) in the Ramachandran design were selected. Improved by 3Drefine server and changed its high (70.4%) and permissible (20.6%) total area (91%). And SWISSMODEL model with the desired allowable area above (97.5%) in the Ramachandran design and the optimal Z score (z-Score=-7.55) from ProSa-web was selected. The quality of the models was evaluated by PROCHECK and ProSa-web servers after modification. Through the Ramachandran diagram, the selected model obtained from PROCHECK, this model was determined in terms of the location of residues in its structure. Also, through the diagram obtained from ProSa-web, the selected model was determined in terms of position among natural patterns in the NMR range (Figure 2).



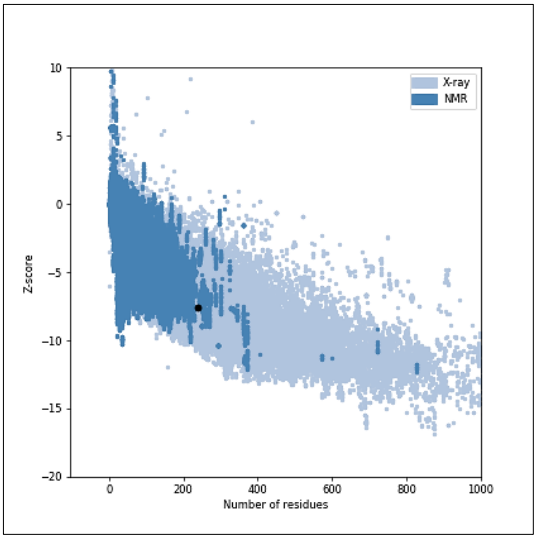
(A)



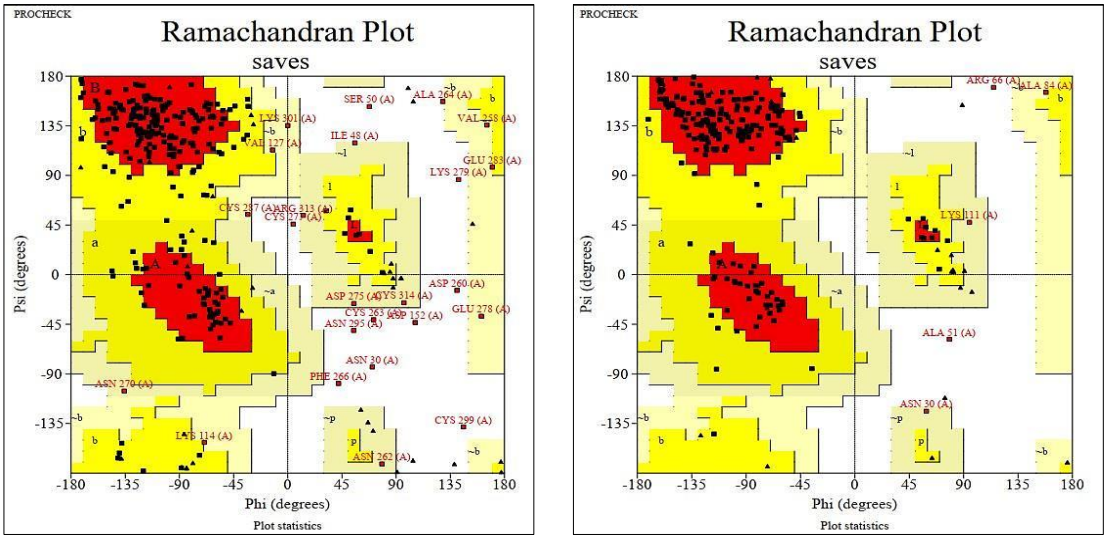
(B)



(C)



(D)



(E) (F)

Figure 2. Figures A and B, respectively, the model image obtained from SWISSMODE and I-TASSER, and figure C and D, respectively, related to the selected models obtained from I-TASSER and SWISSMODE, respectively, in terms of the location of ProSa-web in the NMR approved area. Figures F and E are the evaluation of the correct position of the amino acids of the models obtained from I-TASSER and SWISSMODEL servers, respectively, by the Ramachandran diagram obtained from PROCHECK software.

Allergy prediction results

To assess allergy, ALLERTOP server is a bioinformatics tool that was used to predict the potential allergenicity of the chimeric structure. The method is based on auto cross covariance (ACC) transformation of protein sequences into uniform equal-length vectors. ACC is a protein sequence mining method developed by Wold et al. (Anal. Chim. Acta 1993; 277: 239-253). It has been applied to quantitative structure-activity relationships (QSAR) studies of peptides with different lengths. The principal properties of the amino acids were represented by five E descriptors. They describe amino acid hydrophobicity, molecular size, helix-forming propensity, relative abundance of amino acids, and β-strand forming propensity. The proteins are classified by k-nearest neighbor algorithm (kNN,k=1) based on training set containing 2427 known allergens from different species and 2427 non-allergens.

Docking results

From the results of docking ten three-dimensional models of HER2 receptor and Bkm scFv + chimeric protein were generated by ClusPro server. The immunotoxin protein binded to the HER2 receptor with high affinity and specificity to the HER2 receptor. One of the best illustrated models for this connection with the amount of energy -1057.7, which is shown compared to other models displayed by Pymol software (Figure 3).

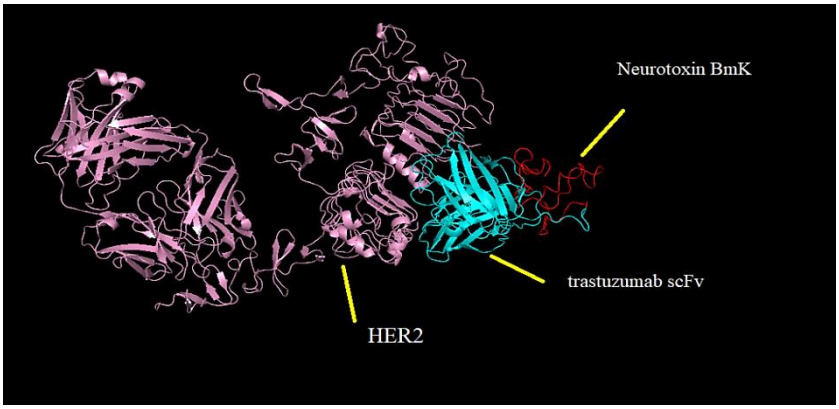


Figure 3. Image of Bkm scFv + docking with HER2 receiver by ClusPro server provided by PYMOL software.

Discussion

Breast cancer is one of the most common cancers known worldwide. Due to the structural heterogeneity of the treatment process, it is associated with many problems. Evidence shows that 25-30% of human HER2 breast cancer cases are positive. This type of cancer is known as the most common malignancy in different races and ages and is the second leading cause of cancer death (13). Numerous studies and observations have shown that conventional breast cancer treatments such as surgery, radiation and chemotherapy have not reduced mortality (14). Among the various types of breast cancer, HER2-positive patients have been found to have a worse prognosis and shorter survival. On the other hand, resistance to chemotherapy is a significant barrier to cancer treatment (15, 16). HER2 has been an attractive target for chemotherapy. Different groups have identified unique sequences that are high affinity for the HER2 receptor, and using modified variants of these sequences that bind to a cytolytic peptide, we have successfully killed cells (17, 18). We have obtained specific HER2-positive cancers. Therefore, HER2 is a receptor with overexpression on the surface of breast cancer cells that is selected for targeted treatment, which as a result of this type of treatment and over the years, the survival of HER2 positive patients has increased (19, 20). The first human anti-HER2 monoclonal antibody is trastuzumab (Herceptin). Only 20% of patients with overexpression of HER2 respond to trastuzumab, according to research. Although it improves the condition of HER2-positive patients and reduces metastasis, there is considerable evidence of trastuzumab resistance (21, 22).

Nowadays, immunotoxin treatment has received a lot of attention due to its specificity and effectiveness on cancer cells. Immunotoxins acquire their toxicity through a protein toxin attached to a portion of specific antibodies, such as monoclonal antibodies (23, 24). Studies have shown that in recent years, several different research groups have observed the anti-cancer effects of raw scorpion venom BmK in vitro or in vivo. Parts of BmK toxin extract cause apoptosis of malignant glioma U251-MG cells in vitro, especially at a dose of 10 mg/ml (25, 26). Another study by Gao et al., (27) showed that BmK toxin can also inhibit the growth of Jurkat and Raji human lymphoma cells by inhibiting cell cycle and causing apoptosis. It is also suspected that compounds isolated from BmK scorpion venom have anti-cancer potential (28). One of them is a serine-like proteinase called BmK-CBP, which can bind dose-dependently to human breast cancer cells MCF-7.31. Another, BmHYA1, a homogeneous hyaluronidase from the BmK scorpion, showed that CD44 expression modulates a cell surface marker in the MDA-MB-231 breast cancer cell line (29, 30). The poison of Buthus martensii Karsch and its extracts have been used for many decades in Asia and some parts of the world to treat cancer and pain. Scorpion, analgesic peptide Buthus martensii Karsch, BmK AGAP belongs to a group of long-chain scorpion peptides and has a molecular mass of 7142 Da with 66 amino acid residues (31-33). BmK AGAP has been reported to have both analgesic and antitumor effects. Today, bioinformatics is changing biology and medicine (34, 35).

In the present study, we used insilico methods to design a specific chimer (immunotoxin), including trastuzumab-derived HER2-specific scFv and a functional part of the neurotoxin toxin in scorpion venom (Bmk), for the treatment of breast cancer patients. This immunotoxin design was similar to the 2017 study by Sokolova et al. (32). They used the scFv fragment of HER2-specific monoclonal antibody "trastuzumab" and PE40, which is part of Pseudomonas a (PE) oxidotoxin, to make a toxic chimera. These chimeric proteins were effective immunotoxins against HER2-positive breast cancer cells in vitro and in vivo. The results of their work confirmed the potent anti-cancer potential of scFv-PE40. In this study, we first generated a scFv using trastuzumab VL and VH and ligated these small antibody fragments via a short peptide linker. In fact, the reason for using scFv was its superior properties, including low molecular weight, less antigen, higher binding and greater specificity compared to antibodies (36).

In fact, VL and VH are joined together by a flexible binder KKKKSKKKKSKSKKKKS to form scFv. The length and composition of the peptide-binding amino acids are important in maintaining the structure and stability of scFv (37). In order to produce an effective anti-cancer immunotoxin, scFv was attached to the Bmk neurotoxin, which is a subunit of the toxin obtained from the venom of buchus scorpions (38). In order to

establish the association between scFv and Bmk, the hydrophobic amino acid GGSGG was selected as the optimal flexible binder. Zhang et al. (36) developed two immunotoxins based on trastuzumab scFv and the cytotoxic drug DM1 called T-SA1-HAS-DM1 and TSA2-HAS-DM1. T-SA1-HAS-DM1 showed potential antitumor activity and was used in xenograft models. Our immunotoxin design was almost identical to T-SA1-HSA. T-SA1-HAS consisted of VL and VH of trastuzumab, which are linked together by the GGGGSGGGGSGGGGS linker. In addition, the linker between scFv and HSA was the GGSGG sequence (38). Recently, Goleij and colleagues developed an immunotoxin containing scFv, the VL and VH of which were linked together by the GGGGSGGGGSGGGGS linker. Their results showed that this scFv is stable and can bind effectively to HER2 (39). Consistent with this study, our scFv was also stable and showed a favorable binding of immunotoxin and HER2. The efficacy of the immunotoxin depends on successful endocytosis, which is based on scFv and receptor interaction and immunotoxin binding to the receptor (40). The immunotoxin-HER2 complex is mediated by endocrine-mediated endocytosis. After internalization, the assemblage can be arranged in primary endosomes or in multicellular bodies. Finally, HER2 is recycled in the membrane and the immunotoxin can go to Golgi (41). Toxic degradation is initiated by the Golgi system of proteins; it then travels to the endoplasmic reticulum (ER) and eventually to the nucleus, causing DNA damage. In line with this pathway and the fact that furine acts as a protease for protein breakdown in the Golgi complex, a protein protein identification site (RGRR amino acid sequence) was placed between the GGSGG binder and HSA. It is predicted that when the scFv + Bmk chimeric protein enters the Golgi, the furin detects this site and breaks the peptide linker, leading to the separation of scFv and Bmk, and that the separation of Bmk can reach the nucleus alone.Weldon et al. Immunotoxins based on Pseudomonas exotoxin A, which contain furin cleavage sites. The results of their study showed that the site of furin degradation is essential for the activation of toxin in the Golgi apparatus and after removal of scFv, the toxin can be released into the cytoplasm (42).

According to the results of this study, in our immunotoxin, when scFv is broken down by the furin protein in Golgi, the toxin can be transported to the ER and then to the nucleus. In the present study, the GORV web server was used to predict the scFv and scFv + Bmk secondary structures. This server estimates the possible secondary structures of proteins by predicting the effect of amino acids on the condition and structure of adjacent amino acids. Comparison between scFv secondary structures and fusion proteins showed no change in their secondary structures when Bmk was attached to scFv via short linkers. Based on the analysis of physicochemical properties, the fusion protein instability index was 36.58, which indicates that the scFv + Bmk protein is stable and its high aliphatic index is related to the stability of the protein over a wide range of temperatures. I-TASSER and SWISSMODEL web servers were used to create a three-dimensional model of the recombinant scFv + Bmk protein in I-TASSE based on a c score. The C score is usually in the range of -5 to +2, and a more positive indicates more confidence in the model and vice versa (43). From the 5 3D models proposed using this server for chimeric protein, the model with the highest c-score was selected for further analysis. After modifying the model structure, evaluation of the modified models was performed using PROCHECK. The results of the Ramachandran design showed the stability of the model.

As shown in the results of the PROMECECK Ramachandran diagram, most of the residues are determined in the desired and desirable regions, and with the modification of the model even, the degree of desirability increases, and this shows that the models in terms of bond and location Amino acids have been improved. These results were confirmed by PROCHECK. Also, the models obtained from SWISSMODEL and I-TASSER were evaluated using software, the resulting Z score of -7 and -7.2, respectively, which indicates the acceptable quality of the models and the location of the resulting models in the NMR range of this server. Fold RNA server used to predict mRNA Secondary structures consistent with minimization of energy obtained. Because the chimeric structure contains Bmk, which is part of the neurotoxin Buthus scorpion venom, it may act as an allergen for humans. Therefore, evaluation of its allergenic ability is required. Allergenic elements stimulate the immune system and lead to adverse allergic diseases (44). The ALLERTOP web server predicted that the chimera protein was not allergenic to the human body. Ligand receptor binding was used to investigate

whether trastuzumab-derived scFv could maintain its binding capacity to the HER2 receptor and transmit Bmk to HER2-positive breast cancer cells. Docking or molecular connection was performed using the ClusPro server. The results of the ClusPro server showed that the scFv + Bmk chimeric protein can bind to HER2 receptors with high affinity and specificity.

Conclusion

New methods of treatment with the help of modern technologies in the field of software are being developed today, based on which we designed a new immunotoxin in this study. Their results showed that scFv + Bmk could be a stable and non-allergenic chimeric protein with a good affinity for increasing HER2 receptors in breast cancer cells. Therefore, the scFv + Bmk structure can be considered a new candidate for immunotoxin against HER2-positive breast cancer. Of course, proving these results requires laboratory and clinical processes.

Acknowledgment

The support of the dr Hamed Esmeil Lashgarian is gratefully acknowledged.

References

1. Jafari SH, Saadatpour Z, Salmaninejad A, Momeni F, Mokhtari M, Nahand JS, Rahmati M, Mirzaei H, Kianmehr M. Breast cancer diagnosis: Imaging techniques and biochemical markers. J Cell Physiol 2018; 233(7): 5200-5213. https://doi.org/10.1002/jcp.26379
2. Lanari C, Wargon V, Rojas P, Molinolo AA. Antiprogestins in breast cancer treatment: are we ready?. Endocr Relat Cancer 2012; 19(3): R35. https://doi.org/10.1530/ERC-11-0378
3. Tan M, Yu D. Molecular mechanisms of erbB2-mediated breast cancer chemoresistance. Breast Cancer Chemosens 2007; 119-29. https://doi.org/10.1007/978-0-387-74039-3_9
4. Loibl S, Gianni L. HER2-positive breast cancer. Lancet 2017; 389(10087): 2415-2129. https://doi.org/10.1016/S0140-6736(16)32417-5
5. Zhou XH, Yang D, Zhang JH, Liu CM, Lei KJ. Purification and N-terminal partial sequence of anti-epilepsy peptide from venom of the scorpion Buthus martensii Karsch. Biochem J 1989; 257(2): 509-517. https://doi.org/10.1042/bj2570509
6. Shao J, Kang N, Liu Y, Song S, Wu C, Zhang J. Purification and characterization of an analgesic peptide from Buthus martensii Karsch. Biomed Chromatogr 2007; 21(12): 1266-1271. https://doi.org/10.1002/bmc.882
7. Gomes A, Bhattacharjee P, Mishra R, Biswas AK, Dasgupta SC, Giri B, Debnath A, Gupta SD, Das T, Gomes A. Anticancer potential of animal venoms and toxins. Indian J Exp Biol 2010; 48: 93–103.
8. Heinen TE, da Veiga AB. Arthropod venoms and cancer. Toxicon 2011; 57(4): 497-511. https://doi.org/10.1016/j.toxicon.2011.01.002
9. Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. J Control Release 2010; 146(3): 264-275. https://doi.org/10.1016/j.jconrel.2010.04.009
10. Liu Y, Xu J, Choi HH, Han C, Fang Y, Li Y, Van der Jeught K, Xu H, Zhang L, Frieden M, Wang L. Targeting 17q23 amplicon to overcome the resistance to anti-HER2 therapy in HER2+ breast cancer. Nat Commun 2018; 9(1): 1-6. https://doi.org/10.1038/s41467-018-07264-0
11. Pal SK, Pegram M. HER2 targeted therapy in breast cancer... beyond Herceptin. Rev Endocr Metab Disord 2007; 8(3): 269-277. https://doi.org/10.1007/s11154-007-9040-6
12. Niu DG, Peng F, Zhang W, Guan Z, Zhao HD, Li JL, Wang KL, Li TT, Zhang Y, Zheng FM, Xu F. Morphine promotes cancer stem cell properties, contributing to chemoresistance in breast cancer. Oncotarget 2015; 6(6): 3963. https://doi.org/10.18632/oncotarget.2894
13. Yang HF, Yu M, Jin HD, Yao JQ, Lu ZL, Yabasin IB, Yan Q, Wen QP. Fentanyl promotes breast cancer cell stemness and epithelial-mesenchymal transition by upregulating α1, 6-fucosylation via Wnt/β-catenin signaling pathway. Front Physiol 2017; 8: 510. https://doi.org/10.3389/fphys.2017.00510
14. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008; 8(10): 755-768. https://doi.org/10.1038/nrc2499
15. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005; 5(4): 275-284. https://doi.org/10.1038/nrc1590

16. Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. Oncogene 2007; 26(45): 6469-6487. https://doi.org/10.1038/sj.onc.1210477
17. Choudhary S, Mathew M, Verma RS. Therapeutic potential of anticancer immunotoxins. Drug Discov Today. 2011; 16(11-12): 495-503. https://doi.org/10.1016/j.drudis.2011.04.003
18. Madhumathi J, Verma RS. Therapeutic targets and recent advances in protein immunotoxins. Curr Opin Microbiol 2012; 15(3): 300-309. https://doi.org/10.1016/j.mib.2012.05.006
19. Mahboobi M, Sedighian H, Hedayati CH, Bambai B, Soofian SE, Amani J. Applying bioinformatic tools for modeling and modifying type II E. coli L-Asparginase to present a better therapeutic agent/drug for acute lymphoblastic leukemia. Iran J Cancer Prev 2017; 10(3): 10. https://doi.org/10.5812/ijcm.5785
20. Goleij Z, Mahmoodzadeh Hosseini H, Amin M, Amani J, Behzadi E, Imani Fooladi AA. In silico evaluation of two targeted chimeric proteins based on bacterial toxins for breast cancer therapy. Int J Cancer Manage 2019; 12(2). https://doi.org/10.5812/ijcm.83315
21. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 2013; 13(10): 714-726.
22. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235(4785): 177-182. https://doi.org/10.1126/science.3798106
23. Urbanelli L, Ronchini C, Fontana L, Menard S, Orlandi R, Monaci P. Targeted gene transduction of mammalian cells expressing the HER2/neu receptor by filamentous phage. J Mol Biol 2001; 313(5): 965-976. https://doi.org/10.1006/jmbi.2001.5111
24. Pero SC, Shukla GS, Armstrong AL, Peterson D, Fuller SP, Godin K, Kingsley-Richards SL, Weaver DL, Bond J, Krag DN. Identification of a small peptide that inhibits the phosphorylation of ErbB2 and proliferation of ErbB2 overexpressing breast cancer cells. Int J Cancer 2004; 111(6): 951-960. https://doi.org/10.1002/ijc.20306
25. Wang WX, Ji YH. Scorpion venom induces glioma cell apoptosis in vivo and inhibits glioma tumor growth in vitro. J Neurooncol 2005; 73(1): 1-7. https://doi.org/10.1007/s11060-004-4205-6
26. Allahyari H, Heidari S, Ghamgosha M, Saffarian P, Amani J. Immunotoxin: A new tool for cancer therapy. Tumor Biol 2017; 39(2): 1010428317692226. https://doi.org/10.1177/1010428317692226
27. Gao F, Li H, Chen YD, Yu XN, Wang R, Chen XL. Upregulation of PTEN involved in scorpion venom-induced apoptosis in a lymphoma cell line. Leuk Lymphoma 2009; 50(4): 633-641. https://doi.org/10.1080/10428190902755505
28. Cao P, Yu J, Lu W, Cai X, Wang Z, Gu Z, Zhang J, Ye T, Wang M. Expression and purification of an antitumor-analgesic peptide from the venom of Mesobuthus martensii Karsch by small ubiquitin–related modifier fusion in Escherichia coli. Biotechnol Prog 2010; 26(5): 1240-1244. https://doi.org/10.1002/btpr.433
29. Zhao Y, Cai X, Ye T, Huo J, Liu C, Zhang S, Cao P. Analgesic-antitumor peptide inhibits proliferation and migration of SHG-44 human malignant glioma cells. J Cell Biochem 2011; 112(9): 2424-2434. https://doi.org/10.1002/jcb.23166
30. Feng L, Gao R, Gopalakrishnakone P. Isolation and characterization of a hyaluronidase from the venom of Chinese red scorpion *Buthus martensi*. Comp Biochem Physiol Part - C Toxicol Pharmacol 2008; 148(3): 250-257. https://doi.org/10.1016/j.cbpc.2008.06.003
31. Taheri-Anganeh M, Khatami SH, Jamali Z, Savardashtaki A, Ghasemi Y, Mostafavi-Pour Z. In silico analysis of suitable signal peptides for secretion of a recombinant alcohol dehydrogenase with a key role in atorvastatin enzymatic synthesis. Mol Biol Res Commun 2019; 8(1): 17. https://doi.org/10.22099/mbrc.2019.31801.1372
32. Sokolova E, Guryev E, Yudintsev A, Vodeneev V, Deyev S, Balalaeva I. HER2-specific recombinant immunotoxin 4D5scFv-PE40 passes through retrograde trafficking route and forces cells to enter apoptosis. Oncotarget 2017; 8(13): 22048. https://doi.org/10.18632/oncotarget.15833
33. Ahmad ZA, Yeap SK, Ali AM, Ho WY, Alitheen NB, Hamid M. scFv antibody: principles and clinical application. Clin Exp Immunol 2012; 2012: 1-15. https://doi.org/10.1155/2012/980250
34. Chen X, Zaro JL, Shen WC. Fusion protein linkers: property, design and functionality. Adv Drug Deliv Rev 2013; 65(10): 1357-1369. https://doi.org/10.1016/j.addr.2012.09.039
35. He Z, Gharaibeh RZ, Newsome RC, Pope JL, Dougherty MW, Tomkovich S, Pons B, Mirey G, Vignard J, Hendrixson DR, Jobin C. Campylobacter jejuni promotes colorectal tumorigenesis through the action of cytolethal distending toxin. Gut 2019; 68(2): 289-300. http://dx.doi.org/10.1136/gutjnl-2018-317200

36. Zhang H, Wang Y, Wu Y, Jiang X, Tao Y, Yao Y, Peng Y, Chen X, Fu Y, Yu L, Wang R. Therapeutic potential of an anti-HER2 single chain antibody–DM1 conjugates for the treatment of HER2-positive cancer. Signal Transduct Target Ther 2017; 2(1): 1-1.
37. Raja SM, Desale SS, Mohapatra B, Luan H, Soni K, Zhang J, Storck MA, Feng D, Bielecki TA, Band V, Cohen SM. Marked enhancement of lysosomal targeting and efficacy of ErbB2-targeted drug delivery by HSP90 inhibition. Oncotarget 2016; 7(9): 10522. https://doi.org/10.5812/ijcm.83315
38. Shilova ON, Proshkina GM, Lebedenko EN, Deyev SM. Internalization and recycling of the HER2 receptor on human breast adenocarcinoma cells treated with targeted phototoxic protein DARPinminiSOG. Acta Nat 2015; 7(3): 126-132. https://doi.org/10.32607/20758251-2015-7-3-126-132
39. Guerra L, Teter K, Lilley BN, Stenerlöw B, Holmes RK, Ploegh HL, Sandvig K, Thelestam M, Frisan T. Cellular internalization of cytolethal distending toxin: a new end to a known pathway. Cell Microbiol 2005; 7(7): 921-934. https://doi.org/10.1111/j.1462-5822.2005.00520.x
40. Takahashi S, Nakagawa T, Banno T, Watanabe T, Murakami K, Nakayama K. Localization of furin to the trans-Golgi network and recycling from the cell surface involves Ser and Tyr residues within the cytoplasmic domain. J Biol Chem 1995; 270(47): 28397-28401. https://doi.org/10.1074/jbc.270.47.28397
41. Weldon JE, Skarzynski M, Therres JA, Ostovitz JR, Zhou H, Kreitman RJ, Pastan I. Designing the furin-cleavable linker in recombinant immunotoxins based on Pseudomonas exotoxin A. Bioconjug Chem 2015; 26(6): 1120-1128. https://doi.org/10.1021/acs.bioconjchem.5b00190
42. Ikai A. Thermostability and aliphatic index of globular proteins. J Biochem 1980; 88(6): 1895-1898. https://doi.org/10.1093/oxfordjournals.jbchem.a133168
43. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinform 2008; 9(1): 1-8. https://doi.org/10.1186/1471-2105-9-40
44. Kelso JM, Greenhawt MJ, Li JT, Nicklas RA, Bernstein DI, Blessing-Moore J, Cox L, Khan D, Lang DM, Oppenheimer J, Portnoy JM. Adverse reactions to vaccines practice parameter 2012 update. J Allergy Clin Immunol 2012; 130(1): 25-43. https://doi.org/10.1016/j.jaci.2012.04.003

How to cite this paper:

Pirmoradi S. Design of a new immunotoxin from a part of scorpion venom for the treatment of breast cancer. Cent Asian J Med Pharm Sci Innov 2022; 2(1): 15-24.