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In-Silico Design, Evaluation of Flic-*Escherichia Coli* and Sonic Hedgehog Protein Mediated HER-2 Antibody-Antigen Complex for Breast Cancer Treatment

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ABSTRACT

Especially, HER2 and Sonic Hedge Hog protein (Hh), an over expressed surface receptor on breast cancer cells, is selected for targeted therapy and also selected TLR-5 immunomodulation. Hence, the current study, we employed in silico procedures to design a specific antibody-antigen complex, comprised of HER2/Hh specific Scfv derived from the Herceptin conjugated Hh, as a targeting molecule and bacterial toxin segment obtained from flagellar antigens FliC (E.coli) were conjugated to HER2/Hh specific Scfv via a flexible linker. A 3D model for this chimeric protein was constructed and its structure, stability, solubility and binding to targeted HER2, Hh and TLR-5 were predicted and, then, evaluated, using in-silico procedures. Analysis showed that the chimeric protein could be a stable and soluble protein and the secondary structure of its parts would not change and the protein had a robust 3D structure that might have a stable mRNA structure and the PyDock server results showed that this antibody/antigen chimeric protein can bind to our proposed three targets such as HER2 and Hh receptors with high affinity and specificity and also good immunomodulatory binding of TLR-5.

Keywords: Herceptin; Flagellar antigens; *Escherichia coli*; Antibody; Antigen complex; *In Silico*

INTRODUCTION

Breast Cancer (BC) is one in all the foremost prevalent sorts of cancer among women worldwide, which is acknowledged because the majority of distinguished reason for cancer mortality. Whole genome expression studies using microarrays have led to classification of BC into five deferent subtypes of breast carcinomas based solely on clustered gene expression; luminal A (ER+ and/or PR+, HER2, low Ki-67), luminal B (ER+ and/or PR+, HER2/+, high Ki-67), HER2-overexpressing (ER+, PR+ and HER2+), basal-like (express markers of basal/myoepithelial cells) and normal breast-like (enriched in markers of adipose cells/normal mammary cells). The role of the Sonic Hedgehog Protein (Hh) pathway in BC tumorigenesis and progression, its prognostic role and its value as a therapeutic target vary in line with the molecular and histological subtype of BC particularly, Hh signaling appears to be a vital mechanism in carcinoma with ER and PR-positive tumors and also Triple Nagative carcinoma TNBC an additional research explored that estrogen increases SHH and GLI1 expression resulting in activation of Hh signaling (determined by GLI1 nuclear translocation) and promotes invasiveness within the ER-positive T47D (HER2-) and BT-474 (HER2+) cells [1]. These results suggest a crosstalk of ER, HER-2 and also the Hh signaling pathways to extend invasiveness of ER-positive BC cells. Many of this treatments for BC-carcinoma, including surgery, radiation and chemotherapy, haven't been effective in diminishing mortality rates, but immunotherapy has become one in all the best approaches to cancer treatment in an exceedingly model of human, HER-2/neu(C) carcinoma (neu-transgenic mice), topical treatment with a TLR7 agonist, imiquimod, showed significant regression of spontaneous breast cancers [2]. Antibody therapies with high efficiency and low toxicity are becoming one of the key approaches in antibody therapeutics. Under this background, the present work, base on high-throughput sequencing and increasing predicted experimental structures of antibody-antigen complexes, computational approaches can predict the antibody/antigen structures and the function of antibodies and design antibody-antigen complexes with improved properties by using non-pathogenic bacterial toxins as a bio-conjugate [3]. Trastuzumab (Herceptin) is one of the humanized monoclonal anti-HER2 antibodies used for targeting HER2-positive breast cancer. It shows better improvement rates during therapy than other treatments do, patients have demonstrated resistance to trastuzumab, even when combined with other chemotherapy drugs [4]. Based on the literatures, it is suggested that treatment with anti-HER2 antibody-targeted toxin is possibly more effective than the anti-proliferative antibody for patients. Although, it has a small antibody fragment composed of heavy (VH) and light (VL) chains, which has to connected to one another through short flexible peptide linkers (about 10-25 amino acids). This has been used as immunotoxins for its low molecular weight (~1 kDa), high elasticity in chemical conjugation, low antigenicity, easy production, great tissue/cell penetration and high biocompatibility, binding and specificity. Trastuzumab (Herceptin) has been extended to transfer

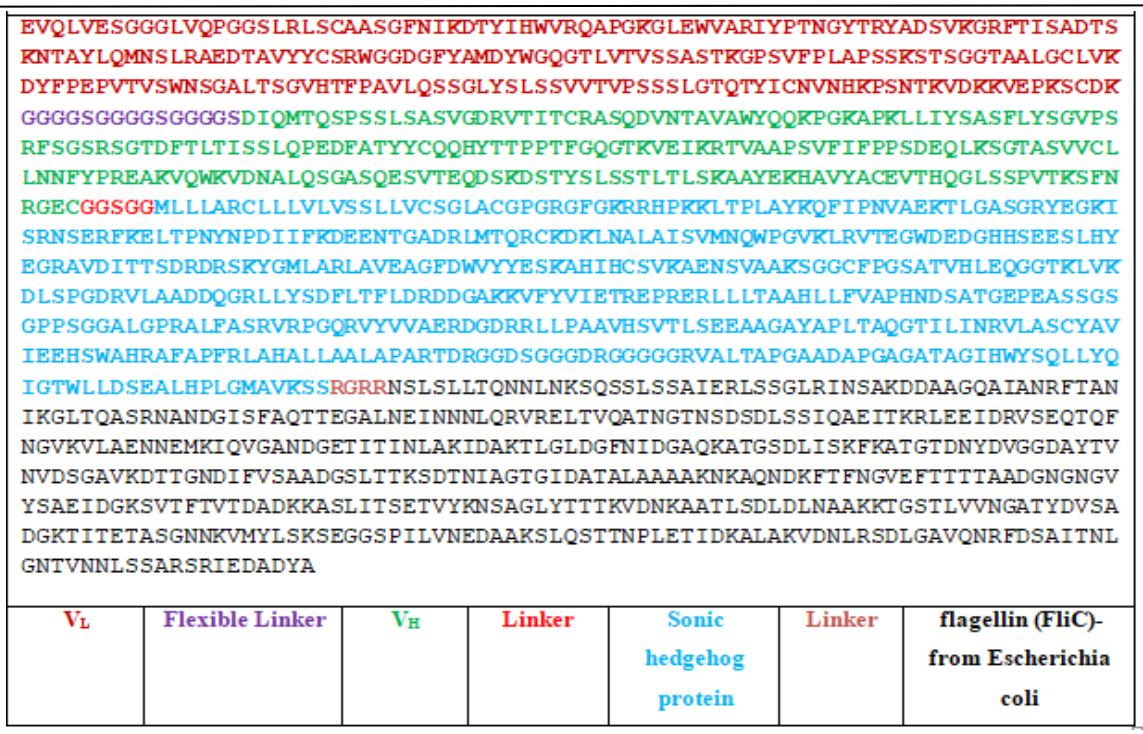

Table 1: Homology model and matching of template and target sequences and resolution.

Template	Seq identity	Oligo-state	QSQE	Found by	Method	Reolution	Seq Similarity	Range
1hzh.1.B	47.44	homo-dimer	0.11	HHblits	X-ray	2.70Å	0.42	1 - 446

Figure 2: 3D-Structure of homology modeled antibody-antigen complex: A). Secondary structure of antibody-antigen complex; B) Refined 3-D structure of our targeted FliC-*Escherichia coli* and sonic hedgehog protein mediated HER-2 antibody-antigen complex, which indicating the green colour shown the sonic hedgehog protein domain, reddish yellow shown the Herceptin-V-Light and V-Heavy chain and White shown the flagellin (FliC)-from *Escherichia coli*.

Stabilization-energy minimization

Energy minimization of targeted antibody/antigen complex were done at Hyperchem was calculate geometry optimizations (minimizations) with molecular and quantum mechanical methods. Geometry optimizations find the coordinates of a molecular structure that represent a potential energy minimum, the minimized structure and energies were shown Figure 3.

successful endocytosis, which is based on scFv and receptor interaction and internalization of the receptor-bound immunotoxin. The immunotoxin-HER2/sonic hedgehog protein mediated antibody complex is internalized *via* receptor-mediated endocytosis [21]. After internalization, the complex can be sorted in early endosomes or in multivesicular bodies. Finally, HER2 is recycled to the membrane and the immunotoxin can go to Golgi. The cleavage of Cytolethal Distending Toxin (CDT) is initiated by the furin protease of the Golgi system; then, it retrogrades to the Endoplasmic Reticulum (ER) and ultimately, it is transported into the nucleus and leads to DNA damage [22]. In accordance with this pathway and based on the fact that furin is enriched in the Golgi complex and acts as a protease for protein cleavage, a furin protease recognition site (RGRR amino acid sequence) was inserted between the GGSGG linker of a Sonic hedgehog protein and the Flagellin (FliC)-from *Escherichia Coli* Segment [23]. It is predicted that when the scFv+Sonic hedgehog protein+flagellin (FliC)-from *Escherichia coli*, chimeric protein enters the Golgi, furin recognizes this site and breaks the peptide linker, which results in the scFv and Sonic hedgehog protein separation and the flagellin (FliC)-from *Escherichia coli* segment could reach the nucleus alone. Weldon designed immunotoxins based on Pseudomonas Exotoxin A that contained furin cleavage sites. Their study results showed the furin cleavage site is necessary for toxin activation in the Golgi apparatus and after scFv omission, the toxin can release to the cytoplasm. In accordance with the mentioned study results, in our immunotoxin of antibody/antigen complex, when scFv cleaved by furin protease in Golgi, the toxin (Sonic hedgehog protein conjugated flagellin (FliC)-from *Escherichia coli*) could be transferred to the ER and then to the nucleus. Sonic hedgehog protein conjugated flagellin (FliC)-from *Escherichia coli* attaches to Herceptin mediated scFv *via* the short linkers (GGSGG), showed no changes in their secondary structures. Based on the analysis of physicochemical features, the Logp, refractive index, polarizability, mass and net-charge for the fusion protein was -448.10, 6033.04 a3, 2535.57A3, 39705.77amu and 0.20e respectively, demonstrating that the Herceptin mediated scFv+Sonic hedgehog protein conjugated flagellin (FliC)-from *Escherichia coli* protein is stable and its supposed high aliphatic index is associated with protein stability across a wide range of temperatures [24]. The swiss-model template library web server was applied to create the 3D model of the Herceptin mediated scFv+Sonic hedgehog protein conjugated flagellin (FliC)-from *Escherichia coli* chimeric protein based on the c-score. C-score is commonly within the range of -5 to +2 and more positivity signifies higher confidence of the model and vice versa. Among the 20 proposed 3D models constructed using this server for the chimeric protein, the model with the highest c-score was selected for further analysis. After the model structure's refinement, the evaluation of unrefined and refined models was performed, using RAMPAGE and PROCHECK. Ramachandran plot results were favor of residues residence before and after the model refinement. According to energy minimization and molecular dynamic study, were analyzed by HyperChem. The data indicated that the E-Potential energy of the thermodynamic Herceptin mediated scFv+Sonic hedgehog protein conjugated flagellin (FliC)-from *Escherichia coli* complex was 2.08E+08, indicating that the chimeric protein mRNA is stable. Since the chimeric construct contains a flagellin (FliC) that is a part of a bacterial genotoxin. The PyDock server results showed that Herceptin mediated scFv+Sonic hedgehog protein conjugated flagellin (FliC)-from *Escherichia coli* complex chimeric protein can bind to our proposed three targets such as HER2 receptors, Sonic hedgehog protein receptor and TLR-5 with high affinity and specificity. The visualization outputs determined that the binding ability of Herceptin mediated scFv+Sonic hedgehog protein conjugated flagellin (FliC)-from *Escherichia coli* complex chimeric protein to its receptor was adequate and the analyzed results confirmed that our targeted antibody/antigen complex shows potential antagonistic effect of HER-2 and Sonic hedgehog protein receptors and it's also confirmed that the immunostimulant effect of TLR-5 through binding and activation.

CONCLUSION

The results pointed towards designed the homology modeled herceptin-scFv/Sonic hedgehog protein containing FliC-*Escherichia coli* mediated antibody-antigen complex was a stable and soluble protein with the appropriate affinity to bind to inhibit the HER2 and Sonic hedgehog protein receptors and can immunomodulate TLR-5 receptors on ER, PR, HER-2 positive breast cancer cells and also act a Trible negative breast cancer cells through binding and activation, Hence, herceptin-scFv/Sonic hedgehog protein containing FliC-*Escherichia coli* mediated antibody-antigen Complex an making it appropriate immunotoxin candidate for breast cancer treatment. Under background of this results could be useful for future experimental studies.

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