Computational Biology and Chemistry 68 (2017) 39–42

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem

Research Article

An *in-silico* approach to find a peptidomimetic targeting extracellular domain of HER3 from a HER3 Nanobody

Z. Pourhashem[b,1], M. Mehrpouya[b,1], N. Yardehnavi[a,b,*], A. Eslamparast[c], F. Kazemi-Lomedasht[d]

[a] Laboratory Science Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[b] School of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran
[c] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran
[d] Biotechnology Research Center, Venom & Biotherapeutics Molecules Lab., Pasteur Institute of Iran, Tehran, Iran

ARTICLE INFO

Article history:
Received 7 August 2016
Received in revised form 1 January 2017
Accepted 2 February 2017
Available online 10 February 2017

Keywords:
HER3
Nanobody
in silico
Docking
Peptidomimetic

ABSTRACT

HER3 is an important therapeutic target in cancer treatments. HER3 Nanobodies (Nbs) are a novel class of antibodies with several competitive advantages over conventional antibodies. A peptidomimetic derived from these Nbs can be considered to be a small peptide mimicking some of the molecular recognition interactions of a natural peptide or protein in a three-dimensional (3D) space, with a receptor that has improved properties.

In this study, we introduce a new approach to design a peptidomimetic derived from HER3 Nb through an *in silico* analysis. We propose that the complementarity determining region (CDR3) of HER3 Nb is large enough to effectively interact with HER3 antigen as well as with the entire Nb. A computational analysis has been performed using Nb models retrieved from SWISS-pdb Viewer 4.1.0 (spdbv) as a target spot and HER3 extracellular domain as its antigenic target to identify the interactions between them by the protein–protein docking method. Detailed analysis of selected models with docked complex help us to identify the interacting amino acid residues between the two molecules. The results of *in silico* analysis show that the CDR3 of HER3 Nb might be used by itself as a peptidomimetic drug instead of the full Nb. HER3 peptidomimetic-derived HER3 Nb may reduce Nb production costs and be used as a substitute for HER3 Nb after further experimental work. The paper demonstrates the feasibility of peptidomimetics designs using bioinformatic tools.

1. Introduction

The human epidermal growth factor receptor 3 (HER3), also called erbB-3 receptor or receptor protein-tyrosine kinase, is an essential component in HER signal transduction systems. HER3 is an anchored cell membrane tyrosine kinase-linked receptor that belongs to the epidermal growth factor receptor (EGFR/ERBB) family (Campbell et al., 2010).

A HER3 molecule consists of three domains—an extracellular ligand binding domain extending from 20 to 643 residues, a transmembrane domain extending from 644 to 664, and an intracellular tyrosine-specific protein kinase-containing domain with no protein kinase activity—extending from nucleotide 665–1342, followed by a carboxy-terminal sequence-containing binding site for PI3-kinases that activates the PI3 K/AKT pathway (Phosphatidyl inositol-3 kinase and protein kinase B), which in turn leads to cellular processes such as cell multiplication and differentiation (Mujoo et al., 2014). HER3 specific-ligand—called neuregulin (NRG)—heterodimerizes the receptor and persuades tyrosine kinase phosphorylation and binding to PI3-kinases, SHC-transforming protein, and other HER3 interacting proteins, consequently activating the PI3 K/AKT and Ras/Raf/MAPK cell signal transduction systems that result in cell multiplication (Mujoo et al., 2014). Over-expression of HER3 is frequently seen in several types of cancers, including malignancies in the breast, ovary, prostate, colon, pancreas, stomach, and oral cavity, squamous cell carcinoma of the head and neck (SCCHN), and lung cancer (non-small cell lung cancer) (Gala and Chandarlapaty, 2014).

In contrast to all EGFR/ERBB families, HER3 lacks protein kinase function, which leads to no homodimerization and allows HER3 to

* Corresponding author at: School of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran.
E-mail address: yardehnavi.najmeh@yahoo.com (N. Yardehnavi).
[1] First authors.

http://dx.doi.org/10.1016/j.compbiolchem.2017.02.001
1476-9271/

restore signaling activity against significant inhibition of other ErbB kinases. Recent studies have shown that HER3 plays a central role in HER2 and other HER family members that arbitrate carcinogenesis. It may also serve as a potential biomarker and target for future cancer diagnosis and therapies (Hynes and Lane, 2005).

Because of the dominant role of HER3 in cancer progression and prognosis, many clinical trials for therapy using HER3 Immunoglobulins are currently being evaluated. Current research on HER3 targeting focuses on HER3-related RNA interference (RNAi), HER3 targeted agents, and intervening factors for the extracellular section and downstream HER3 signal transduction system. As mentioned earlier, unlike other HER family members, HER3 lacks protein kinase catalytic activity and hence cannot be inhibited by protein kinase inhibitors (Jiang et al., 2012; Sithanandam et al., 2005).

A number of antibodies have been developed against the HER3 extracellular ligand-binding domain (ECLBD); some of them are now being subjected to clinical trials such as MM-121, a humanized anti-HER3 monoclonal antibody (Schoeberl et al., 2009), U3-1287, a fully humanized HER3 monoclonal antibody (Jathal et al., 2011; Arnett et al., 2011), MM-111 against both HER2 and HER3 (McDonagh et al., 2012), Pertuzumab an anti-HER2-HER3 antibody that inhibits heterodimerization (Sakai et al., 2007), and MEHD7945 (an anti-HER1-HER3 antibody) (Juric et al., 2015).

Nbs, with a molecular weight of 12–15 kDa, are alternatives for conventional antibodies (150–160 kDa proteins). They may be considered to be the smallest antigen binding sections acquired from camel heavy-chain antibodies (HCAbs) (Muyldermans, 2013). In 1993, Hamers, Casterman, and their colleagues identified a new type of antibodies in camels and llamas that are freed from light chains. They named these antibodies HCAbs, as opposed to conventional antibodies (Hamers-Casterman et al., 1993). Nbs are recombinant antibodies obtained from camel HCAbs by genetic engineering techniques (Desmyter et al., 2015). Nbs (~15 kDa) are much smaller than common antibodies and even Fab fragments (50 kDa) or single-chain variable fragments (~25 kDa). Due to their small size, Nbs can target less accessible epitopes inside living cells. Another benefit of Nbs is their consistency in environmental conditions such as high temperatures or acidic solutions (Hassanzadeh-Ghassabeh et al., 2013).

Nbs are comprised of four framework parts interrupted by three CDRs. It is authorized that each CDR of an Nb can imitate a single loop that retains the full activity of a whole antibody and block its receptor (Desmyter et al., 2001; Bond et al., 2003).

HER3 Nb is a new format of HER3 monoclonal antibodies; it consists of one or more immunoglobulin single variable (ISV). Each ISV of HER3 Nb can specifically bind to the domain II of human HER3 and antagonize HER3 ligand-binding and heterodimerization (Knuehl et al., 2011).

Quite recently, considerable attention has been paid in online databases to protein interactions and associations observed among miscellaneous items. Computational predictions of protein–protein associations have been shown to be favourable for the investigation of intracellular signal transduction systems, modelling of protein structures, and identification of different biochemical processes (Ekins et al., 2007a, 2007b; Akhoon et al., 2010).

In this study, we hypothesize that one or two CDRs of HER3 Nb may act as a whole Nb. Thus, we use *in silico* approaches to analyse the interaction among HER3 extracellular domain and the entire HER3Nb or each HER3Nb CDR. The paper proposes a new approach—to design a petidomimetic drug that is originated from an active CDR on HER3 Nb for efficient HER3 targeting. To our knowledge, this is the first study that suggests a peptidomimetic drug from a whole Nb through bioinformatics analysis.

2. Material and methods

The 621-amino-acid chain of the ErbB3 extracellular domain (PDB entry 1M6B) was selected as the extracellular domain of HER-3.

The primary sequence of NbHER3 (GenBank accession number: HW315065.1) was subjected to IMGT (http://www.imgt.org/3Dstructure-DB/cgi/DomainGapAlign.cgi) to find the CDR fragments (Robinson et al., 2003). The NbHER3 complex structure was attained from both I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) and SWISS homology modelling (http://swissmodel.expasy.org/) using the homologous protein from the anti-HER3 antibody (PDB entry 3P0V) with more than 50% identity. Sequences related to CDRs were assigned to I-TASSER, because of its short length (10 amino acids), in order to build a 3D structure (Zhang, 2008; Schwede et al., 2003). All 3D structures were submitted for energy minimization by spdbv (Kaplan and Littlejohn, 2001). Energy-minimized models were appraised through Ramachandran's map using PROCHECK (a program to investigate the stereo-chemical specifications of protein structures) to rate the anticipated protein frameworks (Laskowski et al., 1993). Docking of HER-3 extracellular domain with the evaluated peptide models was accomplished using Hex8.0 without changing software defaults. The complexes with minimum docking energy were identified to explore the binding site residues of the Nbs involved in interaction with the active residues of the HER-3 (Macindoe et al., 2010). The 3D structures of docked proteins were visualized by the YASARA (Yet Another Scientific Artificial Reality Application) software (www.YASARA.org).

3. Results

Various 3D structures were built using the modeller software, refined by energy minimization, and assessed through the Ramachandran plot using PROCHECK. The structures were selected on the basis of their energy levels, number of amino acids in the allowed region of the Ramachandran plot, and C-score from the I-TASSER server. Table 1 shows the length and molecular weight of peptide models and Table 2 depicts the different minimized energies correlated to modelled structures. C-score is an assurance number for estimating the characteristic of anticipated models using I-TASSER. It is determined on the basis of significance threading model alignments and the convergence of the simulation variables. C-score is particularly seen in the magnitude of −5 to 2; a higher C-score value implies a more valid model and vice versa. More than 90% of the backbone and angles of the modelled structures were in the most favoured regions of the Ramachandran plot. The modelled structures are shown in Fig. 1 and the percentile residue of the HER-2 extracellular domain in various regions of Ramachandran plo tshown in Table 3.

The modelled structures derived from NbHER3 as ligands were docked into the binding cavity of the extracellular domain of HER3 protein as the receptor. The 500 software-generated matches in each and the docked conformations were explored on the basis of

Table 1
Peptide models properties used in the study.

Peptides	Aminoacid counts	Molecular weight (Da)	PI[a]
CDR1	10	1135.20	3.56
CDR2	10	955.00	3.80
CDR3	20	2492.77	8.20
CDR1,2	35	3758.05	4.44
CDR2,3	66	7502.27	6.29
NbHER3-complex	143	15666.33	4.83

[a] Isoelectric point.