Chemico-Biological Interactions 254 (2016) 207–220

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Multi-level structure-based pharmacophore modelling of caspase-3-non-peptide complexes: Extracting essential pharmacophore features and its application to virtual screening

Sivakumar Prasanth Kumar, Prakash Chandra Jha*

School of Chemical Sciences, Central University of Gujarat, Gandhinagar, 382030, Gujarat, India

ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form
1 June 2016
Accepted 6 June 2016
Available online 9 June 2016

Keywords:
Caspase-3
Apoptosis
Structure-based pharmacophore models
Excluded volumes
Güner-Henry score
Enrichment study

ABSTRACT

Enormous caspase-3-non-peptide crystal structures have been developed to study the structural basis of caspase-3 enzyme inhibition using active site directed small molecular design. These complexes have not been explored thoroughly to decipher the essential non-covalent interactions made by crystal ligands. We present here a multi-level analysis of these caspase-3 complexes using structure-based pharmacophore approach wherein numerous candidate pharmacophore hypotheses were assessed for its ability to cover available caspase-3 small molecular inhibitor dataset. The reliability of the resultant pharmacophores was evaluated using three different validation sets comprising focussed caspase-3 inhibitors, focussed + random decoys, and focussed + structurally similar random decoys and its performance was measured by the Güner-Henry (GH) scoring and enrichment statistics. Furthermore, the effect on excluded volumes toward caspase-3 inhibitors mapping was investigated by an iterative deletion in the structure-based models and created optimal structure-based pharmacophore models to enable effective design of caspase-3 small molecular inhibitor design.

1. Introduction

The abrogation of apoptotic events in normal cells may lead to cancer, autoimmune disorders, neurodegenerative diseases, myocardial and liver ischemia, inflammation, osteoarthritis and rheumatoid arthritis in opposed to its essential role in regulating animal development, tissue homeostasis and immune responses [1]. Caspases belong to a family of cysteinyl proteases which promotes apoptosis by triggering various signals via intrinsic and extrinsic activation pathways in the apoptotic cascade and classified based on sequence homology, which determines their specific role in biological activities and substrate specificities, into three categories: initiator, executioner and inflammatory/cytokine related caspases [2–4]. Rupinder et al., 2007 showed twelve Human orthologs have been identified among fourteen reported caspases. Associated with inflammation, caspases-1,-4,-5 and -11 are grouped into one family whereas second family comprises both apoptosis initiator caspases (-2,-8,-9 and -10) and effector caspases (-3,-6 and -7) [5,6]. Known as molecular scissors, these enzymes utilize nucleophilic cysteinyl thiol group to cleave peptide bonds in the DEVD sequence pattern with the specificity of aspartic acid (Asp) at the P_1 position [1,5]. Caspase-3 acts at a downstream level to integrate upstream signals from initiator caspases and almost activated in every apoptosis model, which establishes as the most attractive target to terminate apoptosis in degenerative disorders due to imbalance in the apoptosis process either through inefficient or excessive apoptotic events [2,7,8].

The promotion of caspase-3 as the most promising target to slow down apoptotic response which may prove beneficial for therapeutic interventions in neuro- and immuno- degenerative diseases was supported by numerous experimental studies. Evidences of caspase-deficient cells and cell lines and immune-compromised cell-free extracts have revealed that caspase-3 plays a key executioner role and its inhibition can drastically prevent apoptosis *in vitro* and *in vivo* [9–11]. Further, caspase-3 is recognized as an emerging prominent player in spontaneous and anti-Fas mediated apoptosis identified from the purification of active protease in apoptotic cell models [12,13]. Furthermore, data regarding the application of positional scanning peptide libraries to demonstrate substrate specificities among closely related effector caspases (caspase-3 and -7) indicated that caspase-3 was found to

* Corresponding author.
E-mail address: prakash.jha@cug.ac.in (P.C. Jha).

http://dx.doi.org/10.1016/j.cbi.2016.06.011
0009-2797/

be more promiscuous than caspase-7 and functionally active toward large repertoire of substrates that are cleaved during the terminal phase of apoptotic event [14]. These experimental data collectively confirmed the role of caspase-3 as the principal executioner during the destruction phase of apoptosis.

Caspase-3 has been intensively studied to understand its substrate specificity due to its selective hydrolyzation of cellular proteins that lead to cell death which helped in the design and development of specific inhibitors [15,16]. Enormous studies have been carried out during the past 20 years to determine the three-dimensional structures of caspase-3 in complex with inhibitors among which the majority of the inhibitors falls in peptide classes [17–19]. Caspase-3 exhibit shallow and widely dispersed active site consisting of four sub-sites *viz*. S_1 to S_4 [1,20]. Effective circumventive measures are being taken by pharmaceutical companies to substitute peptide inhibitor characteristics such as poor membrane penetration, short *in vivo* half-life and labile pharmacokinetic properties by chemically reactive small molecules to pursue potent inhibition of caspase-3 activity [1,17–19]. Caspase-3 inhibitors constitute three essential parts: an element for active site recognition, Asp at P_1 position and an electrophilic warhead (e.g. aldehydes, vinyl suplhones, epoxides and chloromethyl ketones) to connect nucleophilic cysteinyl thiol group by covalent linkage [21–23]. Keeping these inhibitors specificity elements of caspase-3 in mind, various studies involved the replacement of P_1-P_3 residues by chemical groups which resulted in the development of numerous diverse peptide-based inhibitors [1,6].

Further, more exclusive chemical inhibitors have been reported to inhibit caspase-3 enzymatic activity and crystallized with caspase-3 recombinant structures to understand the structural basis of inhibition. These collective efforts have generated a myriad of caspase-3-non-peptide inhibitor complexes [1,4,6–8,20,24–26]. Computational caspase-3 inhibitor design through pharmacophore-based virtual screening utilized relatively less receptor details for strategic design unless are docking approach wherein a single template is explored for analyzing the binding efficiency of diverse molecules [4,27]. With progressive efforts on the development of computational inhibitor design strategies and methodological developments towards disease of Human importance [28–30], we present here a multi-level analysis of structure-based pharmacophore models from numerous caspase-3-non-peptide inhibitor complexes and prioritize the prominent pharmacophore feature requirements necessary to map diverse dataset of available small molecular caspase-3 inhibitors. This study helps in narrowing down the most suitable template for structure-based molecular design and assessed its performance by closely inspected three different sets of validation molecules. In addition, we explored the steric unfavourable regions indicated by excluded volumes in the structure-based models and examined its role in relation to the sub-sites of caspase-3 active site thereby creating an optimal pharmacophore model for efficient caspase-3 inhibitor design.

2. Materials and methods

2.1. Dataset and its preparation

The thirteen caspase-3-non-peptide inhibitor complexes [1,7,20,24,26] were retrieved from RCSB Protein DataBank (PDB) [31] and prepared using *Prepare Protein* module of Accelrys Discovery Studio version 4.0.0.13259 (Accelrys Software Inc., USA) [32]. The protein preparation workflow performed the following tasks: insertion of missing atoms, loop regions modelling, removal of alternate conformations and crystallographic waters, assignment of pK_a (acid dissociation constant) values and protonation states to titratable residues according to physiological pH and addition of hydrogens to protein-inhibitor complex. Protein chains that bound to ligand were only retained for further analysis.

2.2. Receptor-ligand pharmacophore models

Structure-based pharmacophore models were developed for each selected caspase-3-non-peptide inhibitor complexes using *Receptor-Ligand Pharmacophore Generation* module of Accelrys Discovery Studio (Accelrys Software Inc., USA) [32]. This protocol generates candidate pharmacophores from the binding ligand features that contain intermolecular non-covalent receptor-ligand interactions. Six different pharmacophore types are currently supported include H bond acceptor (HBA) and donor (HBD), hydrophobic feature (Hydrophobic), aromatic ring (Ring_Aromatic) and positive and negative ionizable centers (Pos_ionizable and Neg_ionizable, respectively) [32]. It was ensured both the protein and ligand constituted complete valence shells prior to modelling. The protocol also added excluded volumes (EVs; size = 1.2 Å) to denote the region of inaccessibility by the bound ligand in the receptor binding site.

We requested maximum 10 candidate pharmacophore models from the protein-ligand complex filtered by the internal scoring function, selectivity score. Selectivity score was calculated from Genetic Function Approximation (GFA) [33] pre-built model developed by training 1544 pharmacophore models [32]. The total number of features, feature types and its inter-feature distance bin values are used as an input to GFA model. We defined the minimum and maximum features in a model to 4 and 6, respectively, to ensure specificity of the candidate pharmacophore in a database search.This feature condition was inspired by the study on successful application of four points or quartet pharmacophore points calibrated for focussed combinatorial library [34], similar to our validation set containing *experimentally verified* caspase-3 small molecular (actives and inactives) inhibitors.The inter-feature and feature-specific distance constraints were employed to perceive pharmacophore features. The maximum distance constraint for features types, ionizable centers, H bond (both site and projection sites) and hydrophobic, were set 5.6 Å, 3.0 Å and 5.5 Å, respectively. We assigned a minimum inter-feature distance of 2.0 Å to select features for candidate pharmacophore generations. The maximum distance for EV was set to 5.0 Å to select protein atoms that were less than this distance from the nearest ligand atoms. The minimum distance of 4.0 Å ensured the selection of protein atoms to avoid steric clashes with the bound ligand [32]. After the candidate model construction, the spatial positions of the features were verified by ligand-pharmacophore mapping technique.

2.3. Validation sets and its curation

Candidate pharmacophores developed from prepared caspase-3-non-peptide inhibitor complexes sorted by selectivity score were validated using a validation dataset. The validation set was compiled from the collection of 2123 caspase-3 inhibitors from BindingDB (database release 2015) [35]. Since the BindingDB dataset comprised peptides, peptidomimetics and small molecular caspase-3 inhibitors, manual refinement was carried out by inspecting literature for capsase-3 small molecular inhibitors. We considered molecules from both inhibitory measures, IC_{50} (half maximal concentration of the inhibitor to inhibit caspase-3) and K_i (inhibitor constant) to allow chemical diversity for validating pharmacophore models. The SMILES format [36] of the chemical data was used to generate 3D conformers by an energy minimization technique involving Dreiding force field [37] (number of iterations = 1000 steps, convergence criterion = 10^{-6} kcal/mol) and