Journal of Structural Biology 179 (2012) 338–346

Contents lists available at SciVerse ScienceDirect

Journal of Structural Biology

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

Enriching the human apoptosis pathway by predicting the structures of protein–protein complexes

Saliha Ece Acuner Ozbabacan [a], Ozlem Keskin [a,*], Ruth Nussinov [b,c], Attila Gursoy [a,*]

[a] Center for Computational Biology and Bioinformatics and College of Engineering, Koc University, Rumelifeneri Yolu, 34450 Sariyer Istanbul, Turkey
[b] Basic Science Program, SAIC-Frederick, Inc., Center for Cancer Research Nanobiology Program, NCI-Frederick, Frederick, MD 21702, United States
[c] Sackler Inst. of Molecular Medicine, Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

ARTICLE INFO

Article history:
Available online 14 February 2012

Keywords:
Apoptosis
Protein–protein interaction
Protein–protein complex
Signaling network
PRISM
3D structure

ABSTRACT

Apoptosis is a matter of life and death for cells and both inhibited and enhanced apoptosis may be involved in the pathogenesis of human diseases. The structures of protein–protein complexes in the apoptosis signaling pathway are important as the structural pathway helps in understanding the mechanism of the regulation and information transfer, and in identifying targets for drug design. Here, we aim to predict the structures toward a more informative pathway than currently available. Based on the 3D structures of complexes in the target pathway and a protein–protein interaction modeling tool which allows accurate and proteome-scale applications, we modeled the structures of 29 interactions, 21 of which were previously unknown. Next, 27 interactions which were not listed in the KEGG apoptosis pathway were predicted and subsequently validated by the experimental data in the literature. Additional interactions are also predicted. The multi-partner hub proteins are analyzed and interactions that can and cannot co-exist are identified. Overall, our results enrich the understanding of the pathway with interactions and provide structural details for the human apoptosis pathway. They also illustrate that computational modeling of protein–protein interactions on a large scale can help validate experimental data and provide accurate, structural atom-level detail of signaling pathways in the human cell.

1. Introduction

Apoptosis was first defined as a general mechanism of controlled cell death which regulates animal cell populations. The term (apoptosis is a Greek word for "falling or dropping off" like leaves from trees) was used to underscore the key aspect of kinetics (Kerr et al., 1972). The recent definition of apoptosis is programmed cell death in multicellular organisms; that is, cells committing suicide by activating an intracellular death program; getting engulfed and digested by macrophages without harming their neighbors. Apoptosis helps in regulation of cell number and size, such as the differentiation of fingers in a developing embryo by the programmed death of cells between them; or removal of infected or damaged cells. Apoptotic processes are regulated by extrinsic (also called as extracellular, cytoplasmic or death receptor-induced) and intrinsic (also called intracellular, mitochondrial or B-cell lymphoma 2 (Bcl-2) controlled) pathways (Ghobrial et al., 2005; Sprick and Walczak, 2004; Strasser et al., 2000).

Central players in signal transduction in both the extrinsic and intrinsic pathways are the caspases (cysteine-dependent aspartate-directed proteases). Caspases are members of the protease family, which are synthesized as inactive precursors or procaspases. Procaspases are activated by proteolytic cleavage by other members of their family in response to inducing signals. Once they are activated and become caspases, they can activate other procaspases by cleaving them. In this manner, initiator caspases, such as procaspase-8 and -10, become activated and cleave the inactive effector caspases, such as procaspase-3. The extrinsic pathway is mediated by the death receptors. These include the Tumor Necrosis Factor receptor (TNF-R; also known as DR1), Fas (TNF receptor superfamily, member 6; also known as CD95, DR2, APO-1) and

Abbreviations: AIF, apoptosis-inducing factor, mitochondrion-associated, 1; Apaf-1, apoptotic peptidase activating factor 1; Bax, BCL2-associated X protein; Bcl-2, B-cell lymphoma 2; Bcl-XL, Bcl-extra large; Bid, BH3 interacting domain death agonist; CASP3, caspase-3; CASP6, caspase-6; CASP7, caspase-7; CASP8, caspase-8; CASP9, caspase-9; CytC, cytochrome C; DISC, death-inducing signaling complex; FADD, Fas-associated via death domain; Fas, TNF receptor superfamily, member 6; FLIP, FLICE/CASP8 inhibitory protein (CASP8 and FADD-like apoptosis regulator (CFLAR)); IAP, baculoviral IAP repeat-containing proteins 2, 3 and 4 (BIRC2, BIRC3 and BIRC4 – also known as XIAP(X-linked inhibitor of apoptosis)); KEGG, kyoto encyclopedia of genes and genomes; MyD88, myeloid differentiation primary response gene 88; PDB, protein data bank; PPI, protein–protein interaction; PRISM, protein interactions by structural matching; RMSD, root mean square deviation; STRING, a search tool for recurring instances of neighboring genes; TNFα, tumor necrosis factor alpha; TNF-R1, tumor necrosis factor receptor superfamily, member 1A; TRADD, TNFRSF1A-associated via death domain; TRAIL, tumor necrosis factor (ligand) superfamily, member 10; TRAIL-R, tumor necrosis factor receptor superfamily.

* Corresponding authors. Fax: +90 212 338 1548.
E-mail addresses: okeskin@ku.edu.tr (O. Keskin), agursoy@ku.edu.tr (A. Gursoy).

1047-8477/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2012.02.002

TNF-related apoptosis-inducing ligand receptors (TRAIL-R1; also known as DR4, APO-2 and TRAIL-R2; also known as DR5) (Portt et al., 2011). Following the activation of the death receptors by death ligands, initiator caspases and death domain (DD)-containing adaptor molecules such as FADD (Fas-associated via death domain) are recruited to the DD of the death receptors. This recruited complex (composed of TRAIL-R, FADD, procaspase-8 and FLIP) is called DISC (death-inducing signaling complex) (Kischkel et al., 1995) and it activates a signaling cascade. Effector caspases are activated by this complex for cleavage of death substrates, e.g. DNA fragmentation factor 45 (DFF45), causing events such as DNA or nuclear fragmentation which trigger apoptosis (Portt et al., 2011).

On the other hand, the intrinsic pathway is initiated by stress signals, such as UV-irradiation, γ-irradiation, DNA damage, and genotoxic stress, causing cytochrome C (CytC) release from the mitochondria. The pro-apoptotic members of the Bcl-2 protein family (Bcl-2-associated X protein (Bax), Bcl-2-associated death promoter (Bad), BH3 interacting domain death agonist (Bid) or Bcl-2 homologous antagonist/killer (Bak)) are required for making the mitochondrial membrane permeable for the release of CytC, whereas the anti-apoptotic members (Bcl-extra large (Bcl-XL) and Bcl-2) inhibit CytC release by blocking pro-apoptotic members (Portt et al., 2011). Released CytC binds to apoptotic protease activating factor 1 (Apaf-1) to form the apoptosome and activate initiator caspase, e.g. procaspase-9, which activates the executioner caspases caspase-3, -6 or -7 (Portt et al., 2011). There is another intrinsic pathway, in which caspases do not function, and it is mediated by a pro-apoptotic protein called apoptosis inducing factor (AIF), which causes DNA fragmentation upon its release from the mitochondria (Susin et al., 1999). The extrinsic and intrinsic pathways of apoptosis are observed to cross-talk via caspase-8, which leads to the initiation of the intrinsic pathway by activating the pro-apoptotic Bcl-2 member Bid (Brunelle and Letai, 2009) and the release of CytC from the mitochondria, in addition to its role of activating caspase-3 and triggering apoptosis in the extrinsic pathway.

Proteins interact with each other specifically and selectively to achieve their biological functions. Consequently, it is crucial to know which proteins interact and how, especially in signaling pathways. Structural studies of the PPIs in the apoptosis network focused on the death receptor signaling (especially the structure of DISC) (Ashkenazi and Dixit, 1998; Bodmer et al., 2000; Carrington et al., 2006; Chinnaiyan et al., 1995; Johnstone et al., 2008; Krueger et al., 2001; Lavrik et al., 2005; Peter and Krammer, 2003; Wajant, 2002), on the mediators (inducing or inhibitory) of the pathway (Chen and Goeddel, 2002; Mihara et al., 2003; Riedl et al., 2001a,b; Yu et al., 2009) and on the general mechanisms of apoptosis regulation (Yan and Shi, 2005).

Considerable information is still missing in the human signaling networks. Large scale analysis by experimental methods such as the yeast two-hybrid system, mass spectrometry, tandem affinity purification, DNA and protein microarrays and phage display have limitations; as such they are complemented by computational methods (Shoemaker and Panchenko, 2007). Computational PPI prediction methods mainly handle the task as a classification problem and solve it via a statistical or machine learning approach. Classification methods determine whether a pair of proteins interact or not, but do not give information about *how* they interact. Structural knowledge of proteins helps: docking methods can predict how proteins interact, *if* it is known that they do. However, docking methods are computationally expensive (Hue et al., 2010) on a large scale and usually need additional biochemical data.

PPIs take place through an interface region formed by the complex of two interacting proteins. The interface region is more conserved than the overall structures of proteins (Caffrey et al., 2004) and structurally and functionally different protein pairs can associate through similar interface motifs (Keskin et al., 2004). Based on these considerations, we proposed a high performance PPI prediction method called PRISM (PRotein Interactions by Structural Matching) (Aytuna et al., 2005; Ogmen et al., 2005; Tuncbag et al., 2011a), which uses interfaces of known protein complexes to predict new potential interactions that can use similar interfaces.

Apoptosis is involved in the pathogenesis of many diseases. If the cells fail to undergo apoptosis, an uncontrolled proliferation rate can cause diseases such as cancer, autoimmune diseases and viral infections (Vaux et al., 1994). On the other hand, accelerated rates of apoptosis may cause diseases that are related to cell loss, such as AIDS (acquired immunodeficiency syndrome), neurodegenerative diseases, ischemic injury and toxin-induced liver disease (Thompson, 1995). It is crucial to know the details of the apoptosis signaling pathway, especially structural details of protein–protein interactions, in order to identify targets and design drugs. So far, not many studies have concentrated on the structures of interacting proteins in signaling pathways, such as apoptosis. In this paper, we aim to predict the structures of the complexes formed by interacting protein–protein pairs in the apoptosis pathway of humans, by using the PRISM algorithm and to figure out the implications of the newly obtained structural network.

2. Materials and methods

PPIs in the human apoptosis signaling pathway, which was adopted from KEGG (Kanehisa and Goto, 2000), are analyzed and new interactions are predicted by using the PRISM algorithm. Below, we describe the PRISM algorithm and the template and the target sets that are used for prediction.

2.1. The PRISM algorithm

PRISM requires two input sets: the template and the target. The template set consists of interfaces extracted from protein pairs that are known to interact (PDB complexes) and the target set comprises of protein chains (PDB chains), the interactions of which we want to predict (Fig. 1). The two sides of a template interface are compared with the surfaces of two target monomers. If regions on the target surfaces are similar to the complementary sides of the template interface, then these two targets are predicted to interact with each other through the template interface architecture.

The prediction algorithm consists of four steps, which are depicted in Fig. 1. In the first step, interacting surface residues of target chains are extracted by using the Naccess (Hubbard and Thornton, 1993) program which calculates the accessible surface area of residues (a residue is accepted as a surface residue if its relative surface accessibility is greater than 5%). In the second step the complementary chains of template interfaces are separated and structurally compared with each of the target surfaces by using the MultiProt structural alignment tool (Shatsky et al., 2004). In the third step the structural alignment results are filtered according to some threshold values. For example, if the template chain has less than or equal to 50 residues, then 50% of the template residues should match the target surface residues; if larger than 50, a 30% match of template to target residues is required. In addition, at least one 'hotspot' residue on the template interface should match one of the hotspots on the target surface. The resulting set of target surfaces are transformed onto the corresponding template interfaces to form a complex. PRISM then checks if following transformation the residues of the target chains collide with those of the complementary target partners. Finally, in the last step, the FiberDock (Mashiach et al., 2009, 2010) algorithm is used to refine the