Poster C-11

Evolution of Death-domain-superfamily in apoptosis pathway

Authors:
Juilee Thakar (*Department of Bioinformatics, University of Wuerzburg and Department of Physics, Pennsylvania state*)
Yogeshwar Kelkar (*Integrated biosciences,Department of Biology, Pennsylvania state university*)
Christoph Borner (*Center for biochemistry and molecular cell biology, University of Freiburg*)
Thomas Dandekar (*Department of Bioinformatics, Wuerzburg*)

Short Abstract: Apoptosis takes place during normal development and pathological conditions. Proteins with domains from death-domain-superfamily are involved in apoptosis pathway regulation. We performed sequence, phylogenetic and structural analysis on domains from this family to look for conserved elements to comment on their role in signaling cascades.

Long Abstract:

Introduction: Apoptosis is necessary for maintaining homeostasis of the cell. It takes place during normal development, pathological conditions and is misregulated during many diseases. Proteins with domains from death-domain-superfamily are involved in regulation of apoptosis pathway at many instances. The death-domain-superfamily includes three domains: death domain (DD), death effector domain (DED) and caspase-recruitment domain (CARD). These domains have conserved structural elements that constitutes of six anti-parallel helix bundles. They domains are involved in homotypic interactions.

DDs are present in receptor and adaptor proteins and are involved in decision making interactions during apoptosis signaling. The phylogenetic analysis also shows that DDs of pro-apoptotic proteins and anti-apoptotic proteins are diverged from each other. DDs of adaptors are more diverged than DDs of receptors*. Our previous studies in DD propose the possibility of the presence of sub-domains (in the previous study we found structural sub-domains in a DD. We extend our analysis to see the availability of such domains in the death-domain-superfamily). We have proposed two exclusive conformation of complexes one of which leads to the cell death and another conformation leads to survival/ proliferation.

Methods: We curated domain sequences in fasta format from SMART version 4 and performed phylogenetic analysis using PHYLIP 3.62. We used CLUSTAL W and seaview for sequence analysis. We used SWISS MODEL to model unknown structures of domains. Further docking program, 3D Dock was used to analyze new interacting surfaces.

Higher level of analysis was performed using HyPhy including calculation of site specific substitution rates. The substitution rates (SRs) of sites which include more than 40% gaps in multiple sequence alignment were omitted.

Results and Discussion: We started the analysis with DDs involved in TNF receptor I (TNFRI) signaling as TNFRI transduce death and survival signals. Since we concentrated on one signaling pathway we could classify DDs and perform structural analysis on few proteins, results of which showed the presence of sub-domains in DDs. We then proposed the model to elucidate the role of DD in the decision making between apoptosis and survival during

TNFRI induced signaling. The results of docking studies performed on five DD-DD complexes show that DD sub-domains of RIP can interact in two exclusive conformation with TRADD leading to either recruitment of CRADD (leading to apoptosis mediated by caspase-2) or NFkB (leading to survival/ proliferation)*.

As DDs are involved in many homotypic interactions we asked how the specificity between interacting partners is maintained. Our studies show that DD of adaptor sequences are more diverged than receptor sequence*. We believe that divergence in the sequences is necessary for maintaining and increasing specificity of interacting partners in such a complicated pathway. Divergence in adaptor DD sequences indicates their important role in maintaining the specificity during interactions. In the apoptosis signaling DD containing adaptor proteins might play decisive role in the recognition of the next protein in the cascade. Whereas less divergence in receptor DDs suggest that DD containing protein which interact with receptor is possibly held in proximity to receptor to interact with it upon activation of the receptor. These interesting results in DDs lead us to extend the analysis to all other members of death-domain-superfamily. The preliminary results in humans show that DED sequences are more conserved than DD and CARD sequences. The average pairwise score (APS) of multiple sequence alignment can be ordered in decreasing APS as: DED (31.53) > CARD (25.89) > DD (19.05) in humans. DD and DED containing proteins contribute in the upper half of the apoptosis pathway and are involved in many decision making interactions during apoptosis signaling. It is interesting that though DED is involved in decision making interactions it has the higher APS than CARD. The first CARD containing proteins that are activated during apoptosis signaling are recognized through DED or other domains. Thus we expected to have highest APS for CARD so we performed more analysis. Phylogenetic analysis shows that CARD (in an unrooted tree 95% bootstrap values are greater than 80 (out of 100)) domains have better defined ancestral sequence than DDs (very low boostrap values) and DEDs (very low boostrap values). This led us to analyze substitution rates and their distribution in these three domains.

Substitution rate calculation shows variation across six helices in DD, DED and CARD. We observed the highest substitution rate (39.76) at position 21 in DD (1st alpha helix), this site was also involved in many DD interactions during docking studies that we performed*. SRs in DED were less than DD SRs, the highest SRs were found at positions 16 and 27 (9.88) (in alpha helix 1 and 2 respectively). We believe that sites that have high substitution rates play important role in the recognition of downstream protein. Standard deviation of SRs can be ordered in decreasing order as: DD (17.60) > DED (2.53) > CARD (0.67). We assumed the SRs of CARD are the basal SRs present in death-domain-superfamily. SRs in CARD domain do not vary a lot which can be explained as CARD domain is not involved in decision making interaction in apoptosis cascade. We could say that sites with SRs greater than basal SRs are involved in the recognition. Further the distribution of SRs strengthens the presence of sub-domains.

Conclusions: Results show that domains involved in decision making interactions in apoptosis signaling cascade have higher variation in substitution rates. Lowest APS and highest standard deviation in DDs in humans shows that these domains are critical for recognition during signaling. This can also be supported by an observation that DD containing proteins are not only present in survival and death signaling cascades but also involved in decision making steps.

* Thakar J, Schleinkofer K, Borner C, Dandekar T. RIP death domain structural interactions implicated in TNF-mediated proliferation and survival. Proteins. 2006; 63(3): 413-423