



Interactions of Mcl-1 With Bim Have Implications For Future Drug Discovery Through Targeted Mutagenesis

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Abstract:

Bcl-2 family of proteins is well known for its role in regulating the rate of apoptosis. They play a vital role in the onset of the adverse condition, Cancer. In this study, we have highlighted the importance of the conserved residue interactions that occur amidst the anti- and pro-apoptotic members of the human Bcl-2 protein family. Mcl-1 is one of the anti-apoptotic proteins belonging to the Bcl-2 family. The mutations that destabilize the Mcl-1 protein could be considered as beneficial since the decreased activity of Mcl-1 will lead to an activated apoptosis that can be one of the approaches to treat the uncontrolled proliferation of cells in case of cancer. Phylogenetic studies of the Mcl-1 protein reveals that its BH domains remain almost conserved in many higher organisms implying a considerable functional similarity among them. The BH3 domain is chosen for further study due to its most conserved nature and to show its docking interaction with Bim, which is a pro-apoptotic BH3 only protein. These interactions on being disturbed due to the unavailability/mutation of Mcl-1 change the apoptotic machinery of the cell. The mutations occurring in these binding sites and their effect on the association of the Pro- and anti-apoptotic proteins will dictate the occurrence of the cancer among the individuals. The study has revealed several important facts related to the binding site of the Mcl-1. This protein when altered due to mutations leads to a drastic change in its interaction with the Bim protein ultimately regulating the apoptosis of the cell.

Keywords: Apoptosis, Bcl-2, Mcl-1, Mutations, Phylogenetics

Introduction:

Apoptosis, or programmed cell death (PCD), is a common and an evolutionarily conserved property of all metazoans ^[1]. Dysregulation of apoptosis can, therefore, contribute to the development of many major diseases including cancer, autoimmunity and neurodegenerative disorders. In most cases, proteins of the caspase family execute the genetic programme

that leads to cell death. Bcl-2 proteins are central regulators of caspase activation, and play a key role in cell death by regulating the integrity of the mitochondrial and endoplasmic reticulum (ER) membranes ^[2]. Bcl-2 proteins have been reported in mammals, and several others have been identified in viruses. Bcl-2 family of proteins can be divided into three groups, i.e., multi domain anti-apoptotic proteins (such as Bcl-2, Bcl-xL, and Mcl-1), multi domain pro-apoptotic proteins (such as Bax, BAK, and Bok), and BH3-only pro-apoptotic members (e.g., Puma, Noxa, Bid, and Bim) ^[3]. The pro-apoptotic BH3-only proteins are the most apical regulators of apoptosis induction ^[4].

Mcl-1 is an anti-apoptotic Bcl-2-family protein that protects cells against death. Structures of Mcl-1, and of other anti-apoptotic Bcl-2 proteins, reveal a surface groove into which the α -helical BH3 regions of certain pro-apoptotic proteins can bind ^[5].

The exact molecular mechanism by which Mcl-1 promotes cell survival is not completely understood but is thought to involve suppression of cytochrome c release from mitochondria, possibly via hetero-dimerization with and neutralization of pro-apoptotic Bcl-2 family proteins, for example, Bim or Bak ^[6].

Unlike Noxa, Bim stabilizes Mcl-1 against degradation. Bim and Noxa BH3 domains may induce distinct conformational changes in Mcl-1, one stabilizing it and the other prompting its destruction ^[7]. The outcome of cell death signaling pathways depends on a complex network involving physical interactions between the pro-survival and pro-death members. Structural studies have revealed that the BH3 domain adopts an alpha helical conformation and that the BH3 domain of a proapoptotic member is able to bind to pro-survival members by occupying a hydrophobic pocket formed by the close proximity of their BH1-3 domains ^[8].

Materials and Methods:

Conservation study: Several tools were employed to identify the homology existing in the sequences of Mcl-1 among various organisms including higher and lower. The tools used were TEXTSHADE, BOXSHADE and Clustal W. The sequences were retrieved from the NCBI database.

Target site identification:

In order to identify the functional domain in the sequences SMART tool was applied. Cast P and Qsite finder were used to identify the amino acids involved in the formation of binding sites within the domain region. To check the mutation prone sites within the binding site, tools like DISOPRED (globplot), RONN, and DISEMBL were used.

Mutation Prediction:

I mutant and SIFT tools were used to analyze the effect of amino acid substitutions within the binding site identified. I mutant would show the amino acids that are susceptible for mutation were as SIFT will dictate the effect of substitution by each amino acid at the target site.

Structural Studies:

The 3Dimensional structure of the protein was retrieved from the Protein Data Bank PDB, The tools used to identify the structure were HH Pred and Phyre from Exspasy server.

Docking Studies:

Argus Lab was used to study the interactions between Mcl-1 an anti-apoptotic protein with its binding partner Bim a pro-apoptotic protein. Several sites within the Mcl-1 that are forming the binding site were targeted to dock with the specified (G14) site in the Bim structure. The energies of all the pair of dockings were analyzed.

Results and Discussion:

Sequence Retrieval:

The FASTA sequences of the Mcl-1 and Bim sequences were retrieved from the Uniprot Database. The Mcl-1sequences of several organisms was retrieved for the homology analysis.

Homology/Conservation Analysis:

All the sequences were subjected for Multiple Sequence Alignment using Texshade and Boxshade tools to identify the regions of similarity within the sequences. The results showed that all the Mcl-1 sequences showed complete similarity in the BH3 domains of their sequences which indicates the potential role of this site in binding and function.

Structure Analysis:

As per the Exspasy server the PDB id of the Mcl-1 was 2PQK.

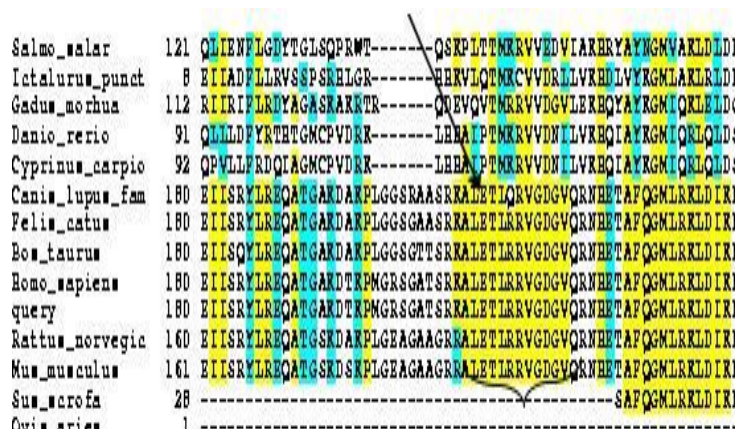


Fig 1: Conservation of the BH3 domain among the sequences of Mcl-1

Binding Site Prediction:

Tool Employed	Binding Sites Predicted
Pocket Finder	209,210,212,213,214,217,218,221
Q Site Finder	209-218
Cast P	210,213,214,217,218,221

Table 1: Binding sites predicted by different tools

Overall residues of importance from the results of CastP, Q-site finder and Pocket-finder are:-A 209,L 210, L 213, R 214, G 217, D 218 but the amino acids showing maximum disorder were only L213,r214,G217 and D218 which were used for further analysis.

I-Mutant results for (LR - - GD) residues.

Position	Amino Acid	Replaced by	Effect on stability
213	L	Any	decrease
214	R	Any	decrease
217	G	E Any other	increase decrease
218	D	E Any other	Increase decrease

Table 2: Effect on stability upon residue replacement

The results showed that all the substitutions in the selected target site were responsible for the decrease in the stability of the protein except in case of two residues 217G and 218D. Substitutions like G217E and D218E are responsible for increasing the stability of the protein. This was further validated using SIFT (D218) to analyze whether these substitutions are tolerable.

SIFT results:

Mcl-1 substitutions for the chosen residues have the following implications

Residue	Tolerated with amino acid residues
L 213	M
R 214	Q,K,L
G 217	S,V
D 218	E,F,G

Table 3: Residues' Tolerance upon substitution

Thus we see that the selected amino acid if replaced by any other residue except the tolerated one can change the normal functioning of the protein. This in turn dictates the rate of Apoptosis.

All the Results obtained from the above analysis were further validated using the BioAxccl^[9] Package a software product of BioAxis DNA Research Centre Private limited that aids the end user to perform the complete gene and protein analysis in a most user-friendly approach and with absolute accuracy.

Docking Analysis:

The optimization of human Mcl-1(2PQKA) and Bim (2PQKB) proteins was done using Sybyl. Docking was performed using the ArgusLab 4.0.1 software.

Mcl-1 residue involved with docking with Bim G14	Best ligand pose energy (first pose) with Bim G14
D 218	-4.32 kcal/mol
G 217	-4.71 kcal/mol
R 214	-4.74 kcal/mol
L 213	-4.28 kcal/mol

Table 4: Docking results of Bim and Mcl-1 variants**Conclusion:**

In the current study, focus was made on the identification of the binding sites within the Mcl-1 protein that are involved in interacting with the pro-apoptotic Bim protein. The rate of formation of these complexes (Mcl-1-Bim) would regulate the apoptosis of the cell. From the results of disorder prediction, the most susceptible mutational sites were identified and the rate of substitution of these residues with the other 19 amino acids was analyzed using I mutant. The effect of these individual substitutions on the stability of the Mcl-1 protein was also calculated which could dictate the rate of dimerization of Mcl1-Bim. The study identified 4 mutational hot spot residues in the binding site of Mcl-1 which are D218, G217, R214 and L213 out of which substitution of L and R with any of the 19 amino acids would decrease the stability of the Mcl-1 were as for the amino acids G and D the substitutions with the amino acid E

increases the stability of Mcl-1 unlike with the other 18 amino acids. Docking was performed between the Mcl1 and Bim proteins at the sites D218, G217, R214 and L213 of Mcl-1 with G14 of Bim. Analysis of the docking results showed that G217 and R214 of Mcl-1 were found to be more energetically favorable; hence targeting these two residues to induce mutation to destabilize the Mcl-1 may be a good approach to increase the rate of apoptosis in cancer cells. Thus, targeted mutagenesis is one of the better approaches to treat cancer.

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