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Designing of Anti-Cancer Drug Targeted to Bcl-2 Associated Athanogene (BAG1) Protein

Amit Kumar, Kriti Verma and Amita Sinha
Department of Bioinformatics and MolecularBiology,
Bioaxis DNA Research Centre, Hyderabad,
India

1. Introduction

Cancer is a disease of uncontrolled cell growth in tissues. This growth may lead to metastasis, which is the invasion of adjacent tissue and infiltration beyond the site of initiation. Cancer is initiated by activation of oncogenes or inactivation of tumor suppressor genes. Nearly 10-30% of all adenocarcinomas are due to the mutations in the *K-ras* proto-oncogene. [1] Function and regulation of Bcl-2 proteins depends upon their interaction with other non-family member proteins, including NIP1, NIP2, NIP3, p53 BP2, Raf-1, CED-4, calcineurin, R-Ras and Bag-1 to form homo and hetero dimmers.[21] Bag1 belongs to the Bcl-2 associated athanogene (BAG) family of multifunctional proteins. This widely expressed protein interacts with a number of signalling molecules (including Bcl2, HGF receptor and Raf1) as it regulates signalling molecules in pathways involving cell survival, growth and differentiation. [13] Bcl2 associated athanogene (BAG1) protein is involved in regulation of the Ras/Raf signal transduction pathway. Of particular relevance to tumour cells, BAG-1 interacts with the anti-apoptotic BCL-2 protein, various nuclear hormone receptors the 70 kDa heat shock proteins, Hsc70 and Hsp70; and serine/threonine kinase. Raf-1 which plays an important role in MAPK pathway.[2][3][4] Recent studies have shown that BAG-1 expression is frequently altered in malignant cells, and BAG-1 expression may have clinical value as a prognostic or predictive marker for various cancer types including breast cancer, prostate cancer and lung cancer.[6][7][8] (Fig 1) Interaction with chaperones may account for many of the pleiotropic effects associated with BAG-1 over expression. The finding that BAG-1 can independently associate with Raf-1 or Bcl-2 provides at least two mechanisms by which BAG-1 promotes cell survival. [20]

Bcl2-associated athanogene (BAG) family proteins participate in a wide variety of cellular processes to regulate growth control pathways, including cell survival (stress response), proliferation, migration, signalling and apoptosis (Fig 2).[2][5][18] This family of co-chaperones functionally regulates signal transduction proteins Raf/MEK/ERK and transcription factors important for cell stress responses, apoptosis, proliferation, cell migration and hormone action. In response to stress, they bind to heat shock proteins HSP70/HSC70 coordinating cell growth signals, by down-regulating the activity of serine/threonine kinase, Raf-1, which plays an important role in MAPK pathway. [5][9] The proteins show anti-apoptotic activity and increase the anti-cell death function of BCL-2 induced by various stimuli. Over expression of BAG-1 suppresses activation of caspases and

apoptosis induced by a very broad range of agents in different cell types, for example chemotherapeutic agents, radiation and growth factor withdrawal. Therefore, in addition to contributing to reduced cell death in cancer development, BAG-1 may also contribute to resistance to important therapeutic modalities.

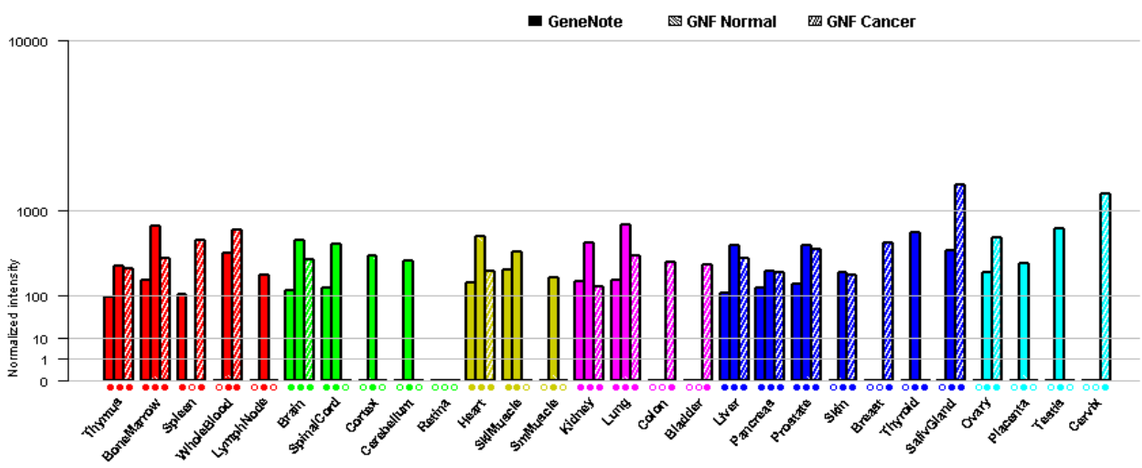


Fig. 1. BAG1 expression in normal and diseased human tissues.

BAG-1 proteins are expressed as multiple isoforms generated by alternate translation initiation from a single mRNA. Translation of the major human BAG-1 isoform, BAG-1S, initiates at an internal AUG codon, whereas of the larger BAG-1L (p50) and BAG-1M proteins translation begins upstream at CUG and AUG codons, respectively.[6][9] Hence, the proteins share a common C-terminus. However, the larger isoforms have additional N-terminal sequences. Various domains have been identified within BAG-1 proteins. [14] A potential nuclear localisation signal (NLS) within the unique N-terminal domain of BAG-1L has been identified. However, BAG-1S and BAG-1M lack this sequence. BAG-1S is largely located in the cytoplasm in contrast to BAG-1M which partitions between the nucleus and cytoplasm. [8]

At the carboxy terminal of all BAG-1 isoforms there is a conserved region of about 110 amino-acids, named as the '**BAG domain',** which binds and regulates Hsp70/Hsc70 molecular chaperones. [8][23] BAG domains are present in Bcl-2-associated athanogene 1 and silencer of death domains.

The crystal structure of the BAG domain revealed that it consists of three anti-parallels α helices. In the BAG domain the first and the second α-helices interact with the serine/threonine kinase Raf-1 and the second and third α-helices interact with the ATP-binding pocket of Hsc70/Hsp70. Therefore, Raf-1 and Hsp70/Hsc70 have partially overlapping sites and their binding is competitive. [2] [8]

BAG-1 promotes cell growth by binding to and stimulating Raf-1 activity. The binding of Hsp70 to BAG-1 diminishes Raf-1 signalling and inhibits subsequent events, such as DNA synthesis, as well as arrests cell cycle. When cellular levels of Hsp70 are elevated during stress, or in cells conditionally over expressing Hsp70, Bag1-Raf-1 is displaced by Bag1-Hsp70, and DNA synthesis is arrested.[5][10] Thus, BAG-1 has been suggested to function as

a molecular switch that controls cells to proliferate in normal conditions but become quiescent under a stressful environment.[16]
The C-terminus of the BAG domain is also a site of interaction with Bcl-2 which provides a supra-additive anti-apoptotic effect. The BAG-1 protein shares no significant homology with Bcl-2 or other Bcl-2 family proteins, which can form homo- and heterodimers. [11]
All BAG-1 isoforms also contain an ubiquitin-like domain (ULD), similar to ubiquitin and ubiquitin-like proteins that appears to be essential for at least some biological effects[8][7][15]. Although the precise function of the ULD in BAG-1 is unknown, BAG-1 isoforms are very stable proteins suggesting that they are not generally targets for degradation by the ubiquitin/proteasome system and are not covalently attached to other proteins.

2. Role of BAG1 and Bcl2 in apoptosis

Bcl-2 is an anti-apoptotic protein located mainly on the outer membrane of mitochondria. It has been found that over-expression of Bcl-2 inhibits cells from undergoing apoptosis in response to a various stimuli. [12] The members of the Bcl-2 family share one or more of the four characteristic domains of homology entitled the Bcl-2 homology (BH) domains (named BH1, BH2, BH3 and BH4).[11][13] The BH domains are known to be crucial for its function, as deletion of these domains via molecular cloning affects survival/apoptosis rates. The anti-apoptotic Bcl-2 proteins, such as Bcl-2 and Bcl-xL, conserve all four BH domains. Bcl-2 interacts with pro-apoptotic proteins BAX and BAK. The hydrophobic unit of Bcl-2 forms a heterodimer with the amphipathic unit of BAX and BAK. This heterodimer formation inhibits release of cytochrome c from the mitochondria and prevents activation of caspases. The protein encoded by BAG1 gene binds to BCL2 and is referred to as BCL2-associated athanogene. It enhances the anti-apoptotic effects of BCL2. [12][13]

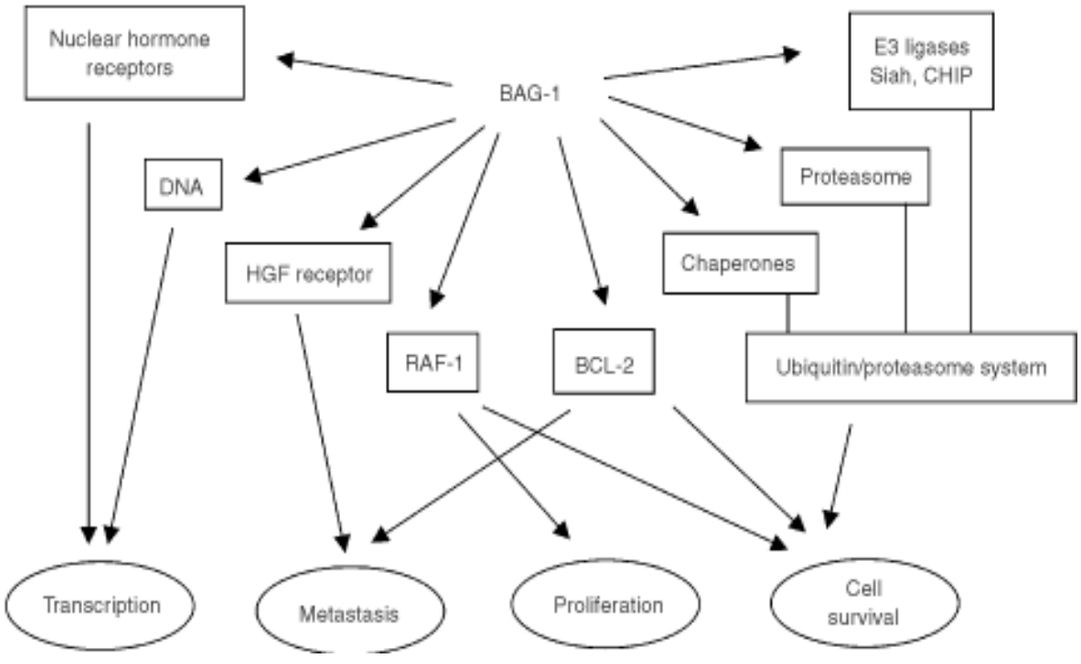


Fig. 2. Interaction of BAG1 protein with other proteins and cellular components. [8]

3. Role of Raf-1/ MAPK pathway in cancer

The pathways regulated by BAG-1 play key roles in the development and progression of cancer and determining response to therapy. The extracellular signal-related kinase (ERK), among the MAPK pathways, plays a key role in promotion of cellular proliferation, survival, and metastasis, this pathway directly affects the initiation and progression of human tumors. This pathway has been found to be activated in numerous cancer types without obvious genetic mutations. Cell lines derived from various organs such as pancreas, colon, lung, ovary and kidney have been reported to show a high degree of MAP kinase activation as observed in 50 tumor cell lines. However, it ihas been found that the constitutive activation of MAP kinases in tumor cells is not due to the disorder of MAP kinases themselves, but is due to the disorder of Raf-1, Ras, or some other signaling molecules upstream of Ras.[3]

The Ras/Raf/MEK/ERK pathway also interacts with the p53 pathway thereby regulating the activity and subcellular localization of BCl2 family proteins (Bim, Bak, Bax, Puma and Noxa). Thus the Raf/MEK/ERK pathway has different effects on growth, prevention of apoptosis, cell cycle arrest and induction of drug resistance in cells of various lineages.[3][4]

4. Methodology used in present study

The strategy used in this project is to target the first alpha helix of the BAG domain. Binding of a ligand to the first alpha helix provides two simultaneous scenarios i.e. firstly it blocks the site for Raf1 binding and thus blocks the MAPK pathway. Secondly, it makes the second and third alpha helix available for Hsp70 binding. Binding of Hsp70 to BAG1 protein renders the heat shock protein inactive as BAG1 has been found to have inhibitory effect on Hsp proteins. This shall produce pseudo stress conditions and attenuate DNA synthesis and cellular proliferation.

Thus, the aim of the present study is to design a drug, targeted to the first alpha helix of 'BAG Domain' of BAG1 protein that binds to the competitive binding site of Raf1 and Hsp70 thereby blocking the binding site of Raf1, making it available for Hsp70 binding and hence suppressing its anti-apoptotic activity. The therapeutic goal is to arrest further tumor progression and trigger tumor-selective cell death by disrupting the balance between pro-apoptotic proteins and anti-apoptotic proteins (Fig 3).

5. Important tools and databases

NCBI is a primary database majorly used for sequence retrieval and similarity based searches. We used NCBI for our sequence retrieval of query protein sequence. BLAST is the most widely used sequence similarity search programme. It finds regions of local similarity between sequences. In this study protein blast has been extensively used. PubMed database was primarily used for literature search including journals, abstracts, full text articles and other sources related to the research. PDB is repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids which is obtained by X-ray crystallography and NMR spectroscopy. PDB was used to retrieve the 3D structure of the protein. Biology Workbench is a web based tool integrated with access to a wide variety of analysis and modeling tools. This tool has been used for phylogenetic analysis of the Bag1 protein sequences.

Clustalw is a multiple sequence alignment program that calculates the best match for the selected DNA or protein sequences and then lines them up so that the identities, similarities and the differences can be seen. Boxshade works by global alignment of all sequence. Conserved and similar residues are emphasized by various degrees of shading. KEGG database was used for pathway analysis for this Bag1 protein. Genecards has been used in this work to get various expression and sequence related information pertaining to proteins of the Bag1 protein. In this work all the above programs have been used to obtain the peptide recognition pattern for the interpretation of results.

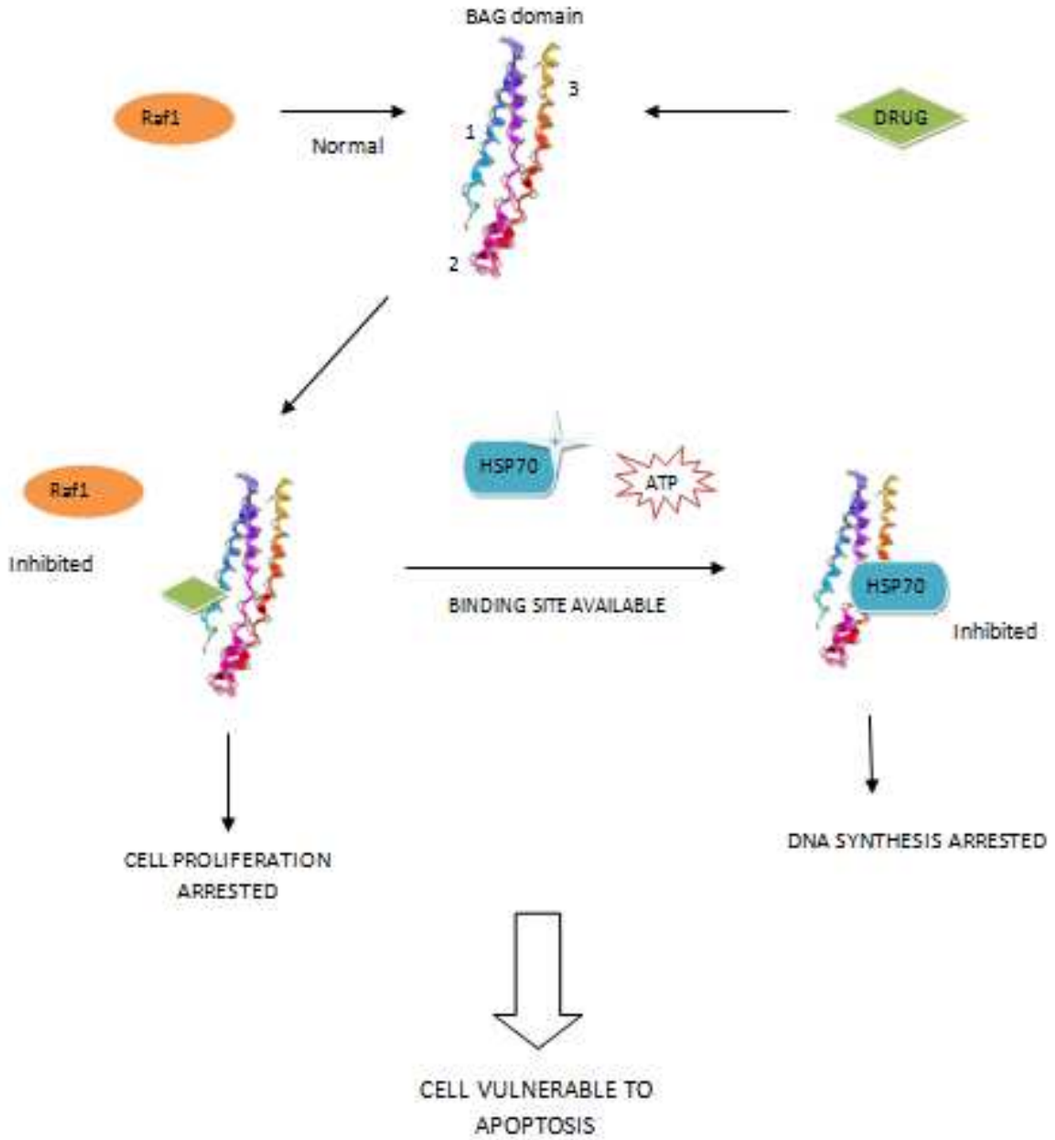


Fig. 3. Flowchart depicting the drug targeted strategy.

6. Structure analysis tools

ProtParam is a tool which allows the computation of various physical and chemical parameters for a protein sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).
The HNN method was used for secondary structure prediction for Bag1 protein. CPHmodels is a web server predicting protein 3D structure by use of single template homology modeling. The CPHmodels server predicts protein structure from amino acid sequence with respect to distance constraints. CPHmodels is a collection of methods and databases consisting of the following tools: CPHmodels was used for tertiary structure prediction of Bag1 protein.
The 3d structure obtained as a pdb file format was viewed using RasMol.
PRODOM and PROSITE is a comprehensive database of protein domain and families. PROSITE offers tools for protein sequence analysis and motif detection.
STRING is a database of known and predicted protein interactions.
CASTP (Computed Atlas of Surface Topography of Proteins) is a server that provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities for proteins and other molecules.

7. Drug discovery tools

Drugbank, Pubchem, therapeutic target database (TTD), Tocris are the various databases which were searched for potential drug candidates.
ArgusLab was used as a molecular modelling program to optimize the target receptor protein and design a drug targeted to the BAG1 protein. The quantum mechanical calculations were performed using the Argus compute server.
HEX5.1 is an interactive protein docking and molecular superposition program. HEX5.1 was used for docking of the selected drug candidate with the BAG1 target protein.

8. Results and discussion

BAG1 isoform 1-L sequence was retrieved from NCBI. The protein BAG1-L is 345 amino acids in length. Sequence analysis by BLASTp shows that Bag1-L protein showed maximum identities with *Bos Taurus* (83%) followed by *Mus musculus* (80%). BLAST results of BAG1 protein compared with other model organisms is shown in Table 1.
Evolutionary relationship of BAG1 protein among various species was obtained in the form of a dendrogram (Fig.4) The query protein was seen to be most closely related to *Mus musculus* and showed a distinct evolutionary relationship with *Suberites domuncula*. The highly conserved regions in the amino acid sequence of BAG1 in protein among various model organisms were analysed using Boxshade (Fig. 5). The amino acids Glycine and Glutamine were found to be the maximum conserved regions among all the species analysed.
The structural analysis of BAG1-L protein was done by using ProtParam, HNN & CPH. Since the GRAVY value is negative (-0.905) it can be inferred from the results that the protein is a hydrophobic molecule present at the cell surface. It is an unstable protein. It consists of 40.00% alpha helices and no beta bridges or turns are present. (Fig. 6) Using CPH model, the 3D structure of the protein was retrieved in the form of a PDB ID. The PDB ID

with the maximum score i.e. 1HX1 was chosen and viewed in Rasmol. (Fig. 7) The PDB ID 1HX1 shows the 3D structure for two molecules Hsp70 and Bag domain as Chain A (400aa) and Chain B (114aa) respectively. The Chain B i.e. Bag domain (receptor) was isolated and its energy was optimized to 3734.78 au using ArgusLab. The molecule converged at 298.92 kcal\mol.

CASTP was used to find the active pocket in the receptor protein. The amino acid Lysine in Chain B (receptor molecule) at position 172 is selected as the target residue for Arguslab docking as it is the most hydrophilic residue in the active pocket.

A number of small molecules that bind to the target were searched by screening libraries of potential drug compounds. The toxic effects and pharmacodynamics of the compounds was tested by ADME/Tox. The compound Carmustine was chosen out of the drug library as it followed the Lipinski's rule of five and had the best combination of required properties for a potential drug candidate. (Table 2)

The molecule Carmustine was designed in ArgusLab and geometry optimization of the drug got converged at energy of 22.2 Kcal\mol. Total energy of the compound converged at -101.413 au.

The drug was docked to the target residue using Arguslab and Hex5.1. In Hex, the drug docked to the receptor with an Emax value (Energy) of -94.68 kcal\mol and Emin value of -166.49 kcal\mol. (Fig. 9) In Arguslab, the drug docked to the target receptor with energy of -5.51 kcal\mol.

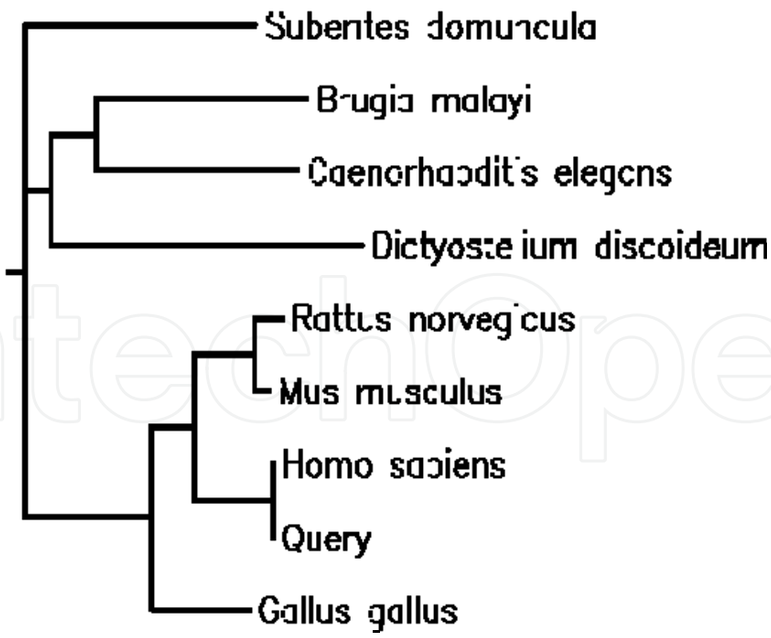


Fig. 4. Dendogram depicting the phylogenetic relationship of Bag1 (Query) protein in Homo sapiens with Bag1 protein of other model organisms (humans).

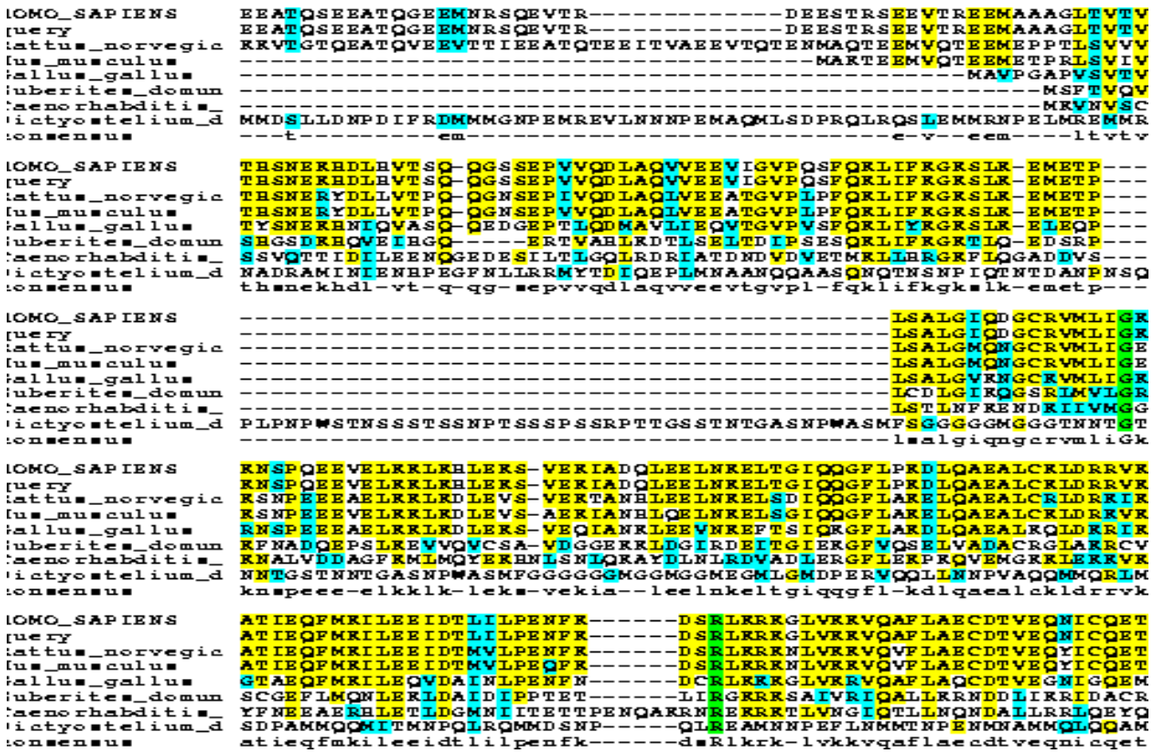


Fig. 5. Boxshade showing some of the highly conserved (green) and similar (cyan) pattern for Bag1 protein in different model organisms.

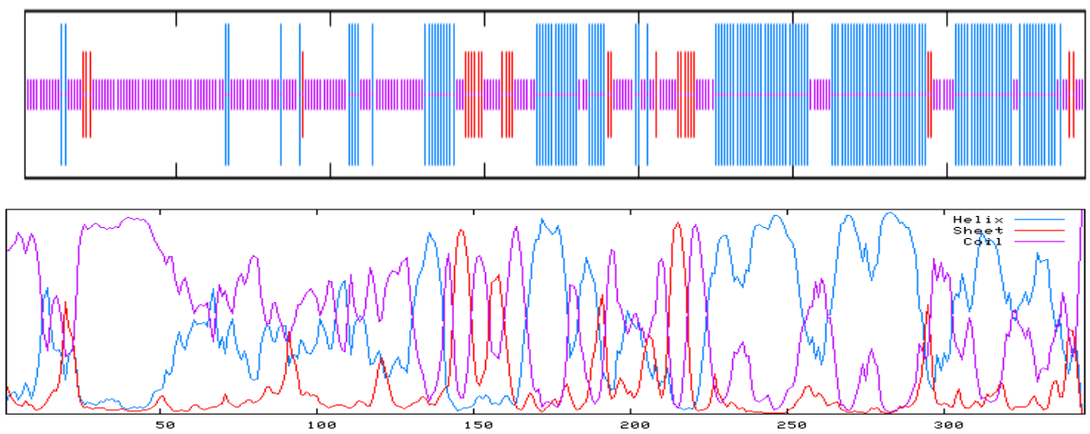


Fig. 6. The secondary structure analysis result of Bag1 by HNN tool. The alpha helices are shaded blue, beta sheets are shaded red.

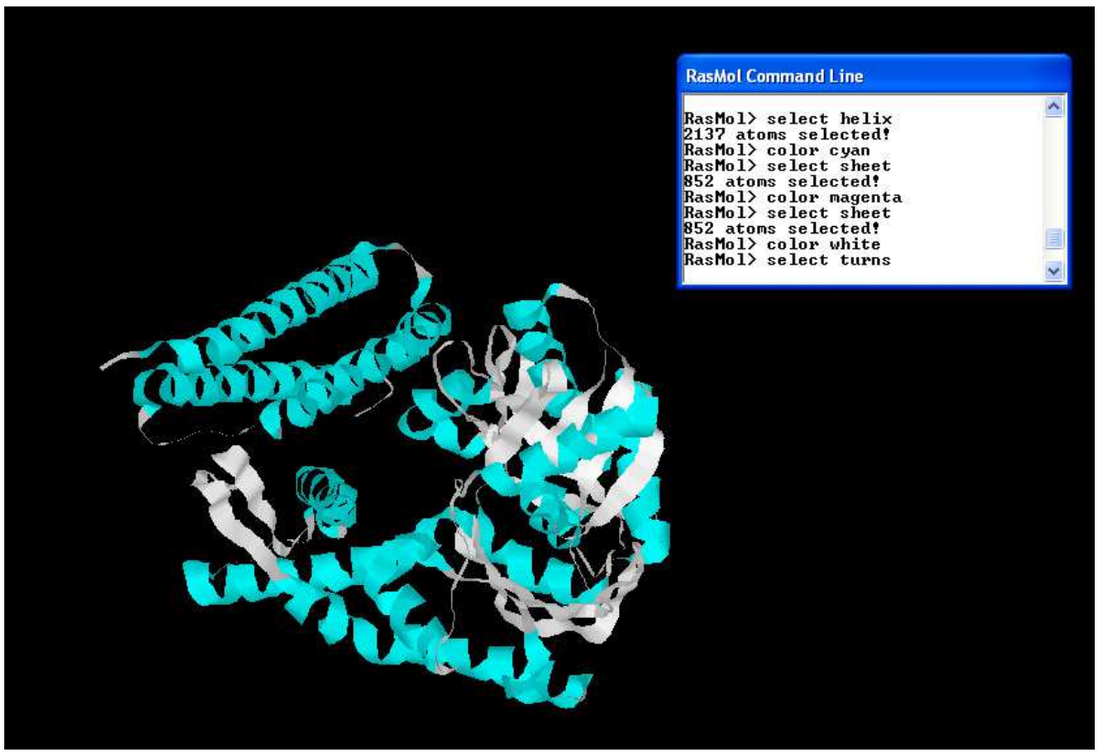


Fig. 7. Visualization result of Bag1protein using the tool RASMOL showing the 3D structure details.

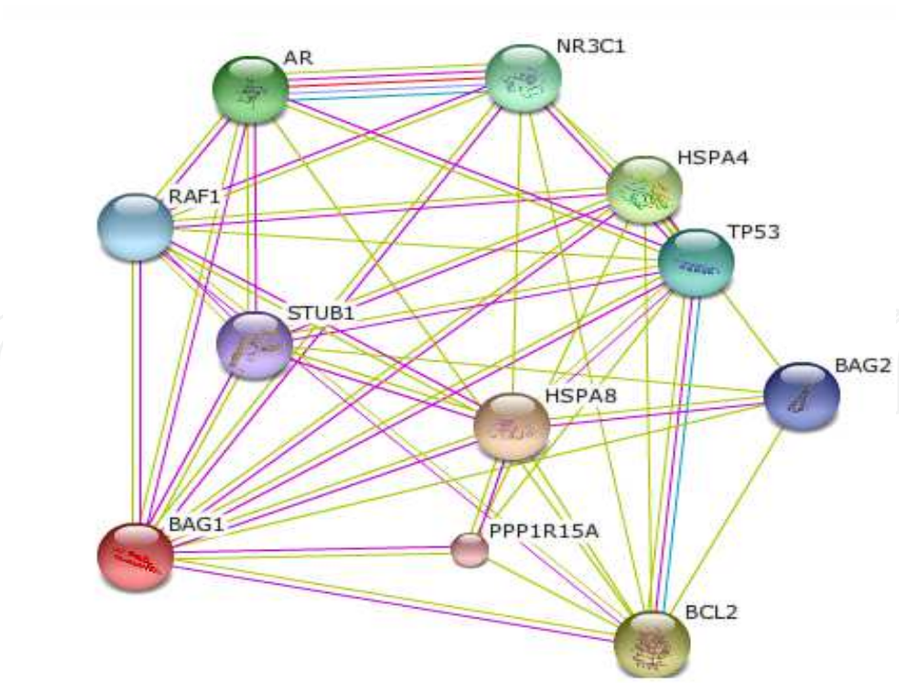


Fig. 8. STRING analysis of BAG1 protein showing its interaction with other proteins in the human. The number of lines represents the strength of interaction.

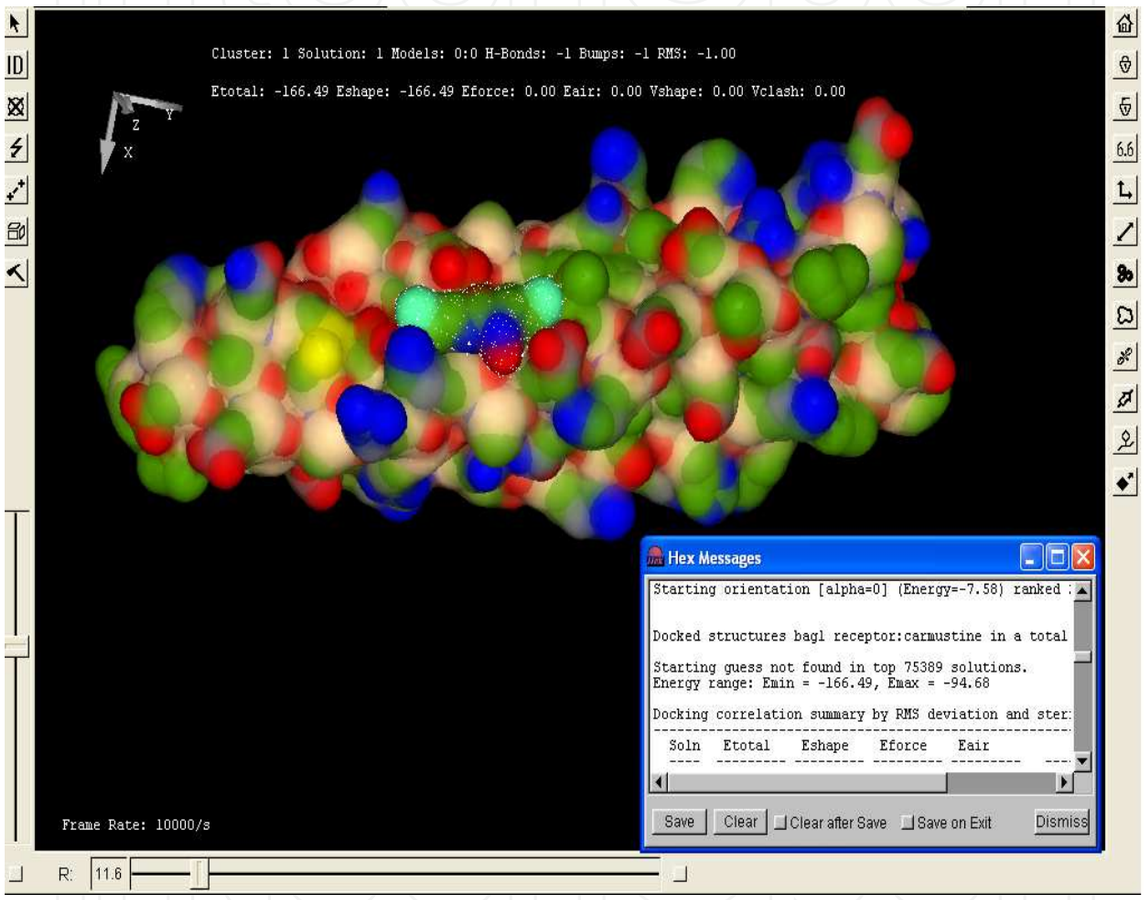


Fig. 9. Docking analysis result of Bag1 protein with the drug Carmustine in Hex5.1.

Model Organism Name	BLAST Results
Homo sapiens	GENE ID: 573 BAG1 | BCL2-associated athanogene [Homo sapiens] (Over 10 PubMed links) Score = 724 bits (1671), Expect = 0.0, Method: Compositional matrix adjust. Identities = 345/345 (100%), Positives = 345/345 (100%), Gaps = 0/345 (0%)
Bos taurus	GENE ID: 613855 BAG1 | BCL2-associated athanogene [Bos taurus] Score = 413 bits (950), Expect = 1e-115, Method: Compositional matrix adjust. Identities = 198/236 (83%), Positives = 214/236 (90%), Gaps = 1/236 (0%)
Mus musculus	GENE ID: 12017 Bag1 | BCL2-associated athanogene 1 [Mus musculus] (Over 10 PubMed links) Score = 362 bits (831), Expect = 1e-99, Method: Compositional matrix adjust. Identities = 172/214 (80%), Positives = 188/214 (87%), Gaps = 0/214 (0%)
Gallus gallus	GENE ID: 420967 BAG1 | BCL2-associated athanogene [Gallus gallus] (10 or fewer PubMed links) Score = 310 bits (712), Expect = 8e-85, Method: Compositional matrix adjust. Identities = 144/209 (68%), Positives = 179/209 (85%), Gaps = 2/209 (0%)
Rattus norvegicus	GENE ID: 297994 Bag1 | BCL2-associated athanogene [Rattus norvegicus] (Over 10 PubMed links) Score = 497 bits (1145), Expect = 9e-141, Method: Compositional matrix adjust. Identities = 250/358 (69%), Positives = 286/358 (79%), Gaps = 13/358 (3%)
Dictyostelium discoideum	GENE ID: 8616246 sonA | UAS domain-containing protein [Dictyostelium discoideum AX4] (10 or fewer PubMed links) Score = 34.5 bits (102), Expect = 0.053, Method: Compositional matrix adjust. Identities = 17/49 (34%), Positives = 29/49 (59%), Gaps = 1/49 (2%)
Caenorhabditis elegans	GENE ID: 172373 bag-1 | BAG1 (human) homolog [Caenorhabditis elegans] (10 or fewer PubMed links) Score = 51.5 bits (160), Expect = 8e-07, Method: Compositional matrix adjust. Identities = 44/145 (30%), Positives = 81/145 (55%), Gaps = 8/145 (5%)

Model Organism Name	BLAST Results
Suberites domuncula	emb|CAJ65915.1| BAG family molecular chaperone regulator 1 [Suberites domuncula] Length=258 Score = 99.5 bits (324), Expect = 4e-19, Method: Compositional matrix adjust. Identities = 63/186 (33%), Positives = 110/186 (59%), Gaps = 4/186 (2%)
Brugia malayi	GENE ID: 6105907 Bm1_55120 | BAG domain containing protein [Brugia malayi] (10 or fewer PubMed links) Score = 47.1 bits (145), Expect = 5e-06, Method: Compositional matrix adjust. Identities = 57/199 (30%), Positives = 92/199 (46%), Gaps = 8/199 (4%)

Table 1. Showing the result of query sequence BLAST with different model organisms.

Drug Name	MW[gm/mol]	LogP	H-Doner	H-Accepter	Hex Docking Value		Arguslab Docking Value[Kcal/mol]
					Emin	Emax	
Carmustine	214.04986	1.53	1	3	-166	-94.68	-5.7995
Benzoyloxy-MAB	331.36	4.75	0	5	-236	-76.51	-9.21813
THIOFLAVIN	226.336	4.59	0	0	-219.48	-94.56	-9.2251
nchembio	283.4116	4.77	0	1	-234.89	-106.75	-8.12071
Disperse Red 53	357.357	2.63	2	7	-245.67	-107.41	-6.99146
Honokiol	266.334	4.95	2	2	-256.14	-92.45	-8.41591
progesterone	314.461	3.89	0	2	-225.06	-80.55	-8.42643
Spectrum_000864	314.4617	3.89	0	2	-226.55	-91.26	-7.82574
CCRIS 4309	373.5289	4.97	3	3	-228.10	-97.30	-7.49083
Methotrexate	432.373601	-1.45	5	4	-288.88	-103.99	-6.4956
Fludarabine	285.231783	<-2	4	9	-198.91	-76.86	-6.4956
2-Fluoroadenosine	285.2317	<-2	4	9	-201.19	-80.10	-6.13115
dexamethasone	392.461063	1.80	3	6	-236.89	-90.34	-7.25208
CID1150209	392.461	1.80	3	6	-223.98	-92.86	-6.94276
Decadronal	434.497713	1.93	2	7	-253.85	-105.54	-7.56959

Table 2. List of various potential drug candidates for binding with Bag domain of Bag 1 protein.

The BAG proteins having anti-apoptotic activity promotes cell growth by binding to and stimulating Raf-1 activity. BAG-1 binds to the serine/threonine kinase Raf-1 or Hsc70/Hsp70 in a mutually exclusive interaction. The binding of Hsp70 to BAG-1 diminishes Raf-1 signalling and inhibits subsequent events, such as DNA synthesis, as well as arrests the cell cycle. Hence Bag1 plays an important role in the progression of cancers when over expressed. The 345 amino acid long protein sequence of BAG1-L was obtained from NCBI and a BLASTp was performed to analyze its evolutionary relationships with other counterparts in various model organisms. This was confirmed by the phylogenetic analysis done using SDSC workbench. The dendrogram presents that Bag1 had close evolutionary relationship with *Mus musculus.*

From the primary structure analysis it was concluded that BAG1 protein is a surface protein which is hydrophilic in nature. Its secondary structure analysis confirmed that it contains more alpha helices and no beta sheets. Its 3D structure was obtained in the form of PDB id and viewed in Rasmol. The chain B of PDB structure represents the BAG domain. Various confirmatory tools were used for validation of the results. The geometry and energy of the BAG domain was optimized in Arguslab. Using Castp, the active pocket in the BAG domain was identified and the most hydrophilic residue in the first alpha helix of the BAG domain i.e. LYS at position 242 of BAG1-L protein sequence obtained from NCBI (or position 172 in the Chain B of pdb id 1HX1) was selected as the target receptor.

A drug library was maintained of possible lead compounds that follow the Lipinski rule of five and their toxicity and disposition was checked using ADME/TOX. These candidate drugs were docked to the target receptor. The drug CARMUSTINE showed the best docking result with docking Energy of -5.51kcal/mol. As the docking (Fig 9) was successful in both HEX5.1 and Arguslab it can be concluded that Carmustine can be a potential drug for BAG1 binding and arresting tumor progression. Further analysis must be performed on this drug for use in treatment of cancer.

9. Acknowledgements

The work was done by worthwhile efforts of the staff and the research associates of the Department of Molecular Biology Bioaxis DNA Research Centre, Hyderabad, India. In addition, the authors would like to thank all the technical staff of instrumental section in developing and maintaining the various databases and tools mentioned in this article.

10. References

[1] Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer.* (2003) 3(1):11-22.

[2] Klára Briknarová1, Shinichi Takayama, Lars Brive, Marnie L. Havert, Deborah A. Knee1, Jesus Velasco1, Sachiko Homma, Edelmira Cabezas1, Joan Stuart, David W. Hoyt, Arnold C. Satterthwait, Miguel Llinás, John C. Reed & Kathryn R. Ely. Structural analysis of BAG1 cochaperone and its interactions with Hsc70 heat shock protein. *Nature Structural Biology,* (2001) 8(4):349-52.

[3] Sebolt-Leopold JS. Oncovera Therapeutics, Ann Arbor. Advances in the development of cancer therapeutics directed against the RAS-mitogen-activated protein kinase pathway. *Clin Cancer Res* (2008) 14:3651-3656

[4] Hoshino R, Chatani Y, Yamori T, Tsuruo T, Oka H, Yoshida O, Shimada Y, Ari-i S, Wada H, Fujimoto J, Kohno M. Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. *Oncogene*. (1999) 18(3);813-22

[5] Sharp A, Crabb SJ, Townsend PA, Cutress RI, Brimmell M, Wang XH, Packham G. BAG-1 in carcinogenesis. *Expert Rev Mol Med.* (2004) 6(7):1-15.

[6] Rudolf Götz, Boris W Kramer, Guadalupe Camarero and Ulf R Rapp. BAG-1 haplo-insufficiency impairs lung tumorigenesis. *BMC Cancer* (2004) 24; 4:85.

[7] HE Maki, OR Saram¨aki, L Shatkina, PM Martikainen, TLJ Tammela, WM van Weerden, RL Vessella, ACB Cato2 and T Visakorpi1. Overexpression and gene amplification of BAG-1L in hormone-refractory prostate cancer. *J Pathol* (2007) 212: 395–401

[8] R I Cutress, P A Townsend, M Brimmell, A C Bateman, A Hague and G Packham. BAG-1 expression and function in human cancer. *British Journal of Cancer* (2002) 87; 834 – 839.

[9] Jaewhan Song, Masahiro Takeda & Richard I. Morimoto. Bag1–Hsp70 mediates a physiological stress signalling pathway that regulates Raf-1/ERK and cell growth. *Nature Cell Biology* (2001) 3: 276 – 282.

[10] Ellen A. A. Nollen,1 Jeanette F. Brunsting,1 Jaewhan Song,2 Harm H. Kampinga,1 and Richard I. Morimoto2 . Bag1 Functions In Vivo as a Negative Regulator of Hsp70 Chaperone Activity. *Mol Cell Biol.* (2000) 20(3):1083-8

[11] Ruth M. Kluck, Ella Bossy-Wetzel, Douglas R. Green, Donald D. Newmeyer. The Release of Cytochrome c from Mitochondria: A Primary Site for Bcl-2 Regulation of Apoptosis. *Science.* (1997) 275(5303):1132-6.

[12] Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science.* (1997) 275(5303);1129-32.

[13] Wang, HG, Takayama S, Rapp UR, Reed JC. Bcl-2 interacting protein, BAG-1, binds to and activates the kinase Raf-1. *Proc. Natl. Acad. Sci. U.S.A* (1996). 93(14):7063-8

[14] Takayama S, Xie Z, Reed JC. An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. *The Journal of Biological Chem.* (1999) 274 (2); 781–786.

[15] Takayama S, Krajewski S, Krajewska M, Kitada S, Zapata JM, Kochel K, Knee D, Scudiero D, Tudor G, Miller GJ, Miyashita T, Yamada M, Reed JC.. Expression and location of Hsp70/Hsc-binding anti-apoptotic protein BAG-1 and its variants in normal tissues and tumor cell lines. *Cancer Res.* (1998) 15; 58(14):3116-31.

[16] Sondermann H, Scheufler C, Schneider C, Hohfeld J, Hartl FU, Moarefi I. Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. *Science.* (2001) 291(5508):1553-7.

[17] Sharp A, Crabb SJ, Johnson PW, Hague A, Cutress R, Townsend PA, Ganesan A, Packham G. Thioflavin S (NSC71948) interferes with Bcl-2-associated athanogene (BAG-1)-mediated protein-protein interactions. *J Pharmacol Exp Ther*. (2009); 331(2): 680–689.

[18] Takahashi N, Yanagihara M, Ogawa Y, Yamanoha B, Andoh T. Down-regulation of Bcl-2-interacting protein BAG-1 confers resistance to anti-cancer drugs. *Biochem Biophys Res Commun.* (2003) 14; 301(3):798-803.

[19] Rorke S, Murphy S, Khalifa M, Chernenko G, Tang SC. Prognostic significance of BAG-1 expression in nonsmall cell lung cancer. *Int J Canc*er. (2001) 20; 95(5):317-22.

[20] Shinichi Takayama, David N.Bimston1, Shu-ichi Matsuzawa, Brian C.Freeman1,2, Christine Aime-Sempe, Zhihua Xie, Richard I.Morimoto1 and John C.Reed3. BAG-1 modulates the chaperone activity of Hsp70/Hsc70. *The EMBO Journal* (1997) 16 ;4887–4896.

[21] Graham P, Matthew B and John LC. Mammalian cells express two differently localized Bag-1 isoforms generated by alternative translation initiation. *Biochem. J.* (1997) 328; 807-813

[22] Yang X, Pater A, Tang SC. Cloning and characterization of the human BAG-1 gene promoter: upregulation by tumor-derived p53 mutants. *Oncogene.* (1999) 12; 18 (32):4546-53.

[23] Zeiner M, Gehring U. A protein that interacts with members of the nuclear hormone receptor family: identification and cDNA cloning. *Proc Natl Acad Sci U S A.* (1995) 5;92(25):11465-9.

[24] Moriyama T, Littell RD, Debernardo R, Oliva E, Lynch MP, Rueda BR, Duska LR. BAG-1 expression in normal and neoplastic endometrium. *Gynecol Oncol.* (2004) 94(2):289-95.

[25] Clemo NK, Collard TJ, Southern SL, Edwards KD, Moorghen M, Packham G, Hague A, Paraskeva C, Williams AC. BAG-1 is up-regulated in colorectal tumour progression and promotes colorectal tumour cell survival through increased NF-kappaB activity. *Carcinogenesis.* (2008) 29(4):849-57

[26] Clemo NK, Arhel NJ, Barnes JD, Baker J, Moorghen M, Packham GK, Paraskeva C, Williams AC. The role of the retinoblastoma protein (Rb) in the nuclear localization of BAG-1: implications for colorectal tumour cell survival. *Biochem Soc Trans.* (2005) 33(Pt 4):676-8.

[27] Townsend PA, Cutress RI, Sharp A, Brimmell M, Packham G. BAG-1 prevents stress-induced long-term growth inhibition in breast cancer cells via a chaperone-dependent pathway. *Cancer Res.* (2003) 15; 63(14):4150-7.

[28] Kikuchi R, Noguchi T, Takeno S, Funada Y, Moriyama H, Uchida Y. Nuclear BAG-1 expression reflects malignant potential in colorectal carcinomas. *Br J Cancer.* (2002) 4; 87(10):1136-9.

[29] Liu HY, Wang ZM, Bai Y, Wang M, Li Y, Wei S, Zhou QH, Chen J. Different BAG-1 isoforms have distinct functions in modulating chemotherapeutic-induced apoptosis in breast cancer cells. *Acta Pharmacol Sin.* (2009) 30(2):235-41.

[30] Vora HH, Mehta SV, Shah KN, Brahmbhatt BV, Desai NS, Shukla SN, Shah PM. Cytoplasmic localization of BAG-1 in leukoplakia and carcinoma of the tongue: correlation with p53 and c-erbB2 in carcinoma. *Int J Biol Markers.*(2007) 22(2):100-7.

[31] Pusztai L, Krishnamurti S, Perez Cardona J, Sneige N, Esteva FJ, Volchenok M, Breitenfelder P, Kau SW, Takayama S, Krajewski S, Reed JC, Bast RC Jr, Hortobagyi GN. Expression of BAG-1 and BcL-2 proteins before and after

neoadjuvant chemotherapy of locally advanced breast cancer. *Cancer Invest.* (2004);22(2):248-56.

[32] Tang SC. BAG-1, an anti-apoptotic tumour marker. *IUBMB Life.* (2002) Feb; 53(2):99-105.

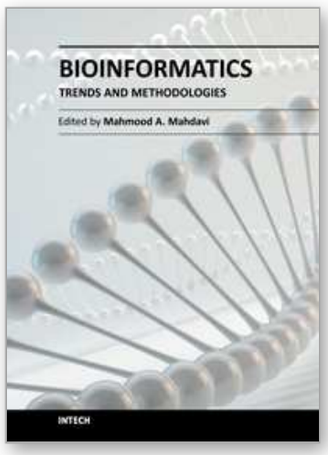
[33] Brive L, Takayama S, Briknarová K, Homma S, Ishida SK, Reed JC, Ely KR. The carboxyl-terminal lobe of Hsc70 ATPase domain is sufficient for binding to BAG1. *Biochem Biophys Res Commun.* (2001) 21; 289(5):1099-105.

[34] Nadler Y, Camp RL, Giltnane JM, Moeder C, Rimm DL, Kluger HM, Kluger Y. Expression patterns and prognostic value of Bag-1 and Bcl-2 in breast cancer. *Breast Cancer Res.* (2008) 10(2):R35.

[35] Lüders J, Demand J, Höhfeld J. The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. *J Biol Chem.* (2000) 275(7):4613-7.

[36] McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A, Stivala F, Libra M, Basecke J, Evangelisti C, Martelli AM, Franklin RA. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochim Biophys Acta*. (2007). 1773(8):1263-84.

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Bioinformatics - Trends and Methodologies
Edited by Dr. Mahmood A. Mahdavi

ISBN 978-953-307-282-1
Hard cover, 722 pages
Publisher InTech
Published online 02, November, 2011
Published in print edition November, 2011

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Amit Kumar, Kriti Verma and Amita Sinha (2011). Designing of Anti-Cancer Drug Targeted to Bcl-2 Associated Athanogene (BAG1) Protein, Bioinformatics - Trends and Methodologies, Dr. Mahmood A. Mahdavi (Ed.), ISBN: 978-953-307-282-1, InTech, Available from: http://www.intechopen.com/books/bioinformatics-trends-and-methodologies/designing-of-anti-cancer-drug-targeted-to-bcl-2-associated-athanogene-bag1-protein

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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