© 2017 IJSRSET | Volume 3 | Issue 5 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section: Engineering and Technology

Comparative Proteomics of Lymphoid Tumour

Kavitha, Dr. C. S. Arunaman

[1]Assistant Professor, PG and Research Department of Zoology, Quaid-E-Millath Government College for Women (Autonomous) , Chennai, Tamil Nadu, India
[2]Professor, Department of Clinics and Jurisprudence Madras and Veterinary and Animal Science University Chennai, Tamil Nadu, India

ABSTRACT

Comparative analysis of lymphoid tumor protein was conducted in seven protein sequences in the present investigation. The protein sequences were retrieved from NCBI data base for BCL-2, BCL-6, BCL-10, BCL-11. BCL-W, BCL-XL and CD-20. Protein sequence was analysed using Bioinformatics tools such as PROTPARAM for identification of physical and chemical parameters, SOPMA for secondary structure prediction, Clustal W for Multiple sequence analysis, MEGA for constructing Phylogenetic tree were used. Molecular weight, Isoelectric point, instability index, number of amino acids of the seven protein sequences were found and analyzed using PROTPARAM. The number of amino acids present in a protein sequence determines the molecular weight of proteins. Hence the protein BCL-6 weighed more than the other proteins (1040860.0 kDa). The instability Index showed that the protein BCL-W is the stable protein which has the value of less than 40 (33.17). All the other proteins are unstable. The secondary structure predication of the protein was analyzed for alpha helix, Beta sheet, coil region and sequence length to analyze seven protein sequences. An interesting finding of multiple sequence alignment in that the amino acid proline was present in all the proteins of lymphoid tumor and in the species taken for the study. From this it could be inferred that the amino acid proline might be responsible in causing lymphoid tumor. More research needs to be done to target proline amino acid to find out an effective and efficient drug candidates be cure lymphoid tumor. Phylogenetic analysis was carried out and the evolutionary relationship was analyzed. The Bootstrap value was used to predict the reliability of the phylogenetic tree constructed. The phylogenetic tree of the CD-20 showed that the species, Mouse and Monkey are closely related. Cattle and Rat are closely related. Horse and Dog are closely related. This node is somewhat closely related with human. The results of this research work could be utilized for further detailed investigations on the seven proteins involved in lymphoid tumor to formulate measures for the betterment of mankind.

Keywords : BCL Protein, NCBI, SOPMA, PROTPARAM

I. INTRODUCTION

Cancer is one of the most dreadful diseases which are globally distributed among the world's population. Cancer occurs due to excessive free radical damage, which ultimately causes damage to the genetic material DNA, protein and lipids. This DNA damage leads to mutations that cause normal cells to transform into a cancer cell [1]. In cancer cells, increased levels of reactive oxygen species have been found, which causes uninterrupted cell proliferation and leads to tumour development [2]. The chances of surviving the disease vary greatly by the type and location of the cancer and the extent of disease at the start of treatment. While cancer can affect people of all ages, and a few types of cancer are more common in children, the risk of developing cancer generally increases with age. In 2008, approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers) and 7.6 million people died of cancer worldwide. Cancers as a group account for approximately 13% of all deaths each year with the most common being: lung cancer (1.4 million deaths), stomach cancer (740,000 deaths), liver cancer (700,000 deaths), colorectal cancer (610,000 mdeaths), and breast cancer (460,000 deaths). This makes invasive cancer the leading cause of death in the developed world and the second leading cause of death in the developing world

[3]. The three most common childhood cancers are leukemia (34%), brain tumors (23%) and lymphoma (12%). Rates of childhood cancer have increased by 0.6% per year from 1975 to 2002 in the United States and by 1.1% per year between 1978 and 1997 in Europe. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals (or) infectious agents. Other cancer promoting genetic abnormalities may randomly occur through errors in DNA replication (or) are inherited and thus present in all cells from birth, the heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome [4-6].

Lymphoma shortly defined is a type of cancer involving cells of the immune system, called lymphocytes. It affects the cells that play a role in the immune system and primarily represents cells involved in the lymphatic system of the body. Generally the lymph nodes filter the lymph, which may on various occasions carry different microbial organisms. At infections sites, large numbers of these microbial organisms, collect in the regional nodes and produce swellings and tenderness. Typical of a localized infection. These enlarged and occasionally confluent collections of lymph nodes (so called lymphadenopathy) are often referred to as 'swollen glands'. These swollen glands cause lymphoid tumors like, Hodgkin's disease, Non-Hodgkin's lymphoid tumor, Primary central nervous system lymphoid tumor. Hodgkin's lymphoma previously known as Hodgkin's disease is a type of lymphoma, which is a cancer originating from white blood cells called lymphocytes. It was named after Thomas Hodgkin, who first described abnormalities in the lymph system in 1832. Hodgkin's lymphoma is characterized by the orderly spread of disease from one lymph node group to another and by the development of systemic symptoms with advanced disease [7]. There are several proteins involved in the lymphoid tumor. Some of the important proteins are BCL-2, BCL-6, BCL-10. BCL-11, BCL-XL, BCL-W and CD.

The Bcl-2 family of proteins play a major role in cancer development The protein BCL-2 is located at 18q21, location_base_pair: Starts at 58941559 and ends at 59137593 bp from pter. The protein BCL-6 is located at 3q27, location_base_pair: Starts at 188921859 and ends at 188936979 bp from pter. The protein BCL-10 is located at 1p22.3, location_base_pair: Starts at 85504048 and ends at 85516171 bp from pter. The protein BCL-11 is located at 2p13-15, location_base_pair: Starts at 60531806 and ends at 60634137 bp from pter. The protein BCL-W is located at 14q11.2, location_base_pair: Starts at 22845852 and ends at 22850808 bp from pter. The protein BCl-XL is located at 11q13.1. The protein CD20, (Synonyms: MS4A1, B-lymphocyte antigen CD20) is a protein, that in humans encodes by the MS4A1 protein [8-10]. Hence the present study was undertaken to elucidate the protein sequences related with lymphoid tumor of different species using bioinformatics tools.

II. METHODS AND MATERIAL

In the present study, to retrieve the protein sequence (BCL-2, BCL-6, BCL-10, BCL-11, BCL-XL, BCL-W and CD) related with lymphoid tumor of different species was analyzed and to perform the comparative analysis and construct phylogenetic tree using bioinformatics tools.

NCBI

NCBI refer to national centre of biotechnology information. The NCBI has had responsibility for making available the GenBank DNA sequence database since 1992. GenBank coordinates with individual laboratories and other sequence databases such as those of the European Molecular Biology Laboratory (EMBL) and the DNA Data Bank of Japan (DDBJ).Since 1992, NCBI has grown to provide other databases in addition to GenBank.

PROTPARAM

ProtParam (References / Documentation) is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered 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) (Disclaimer)

SOPMA

SOPMA (Self-Optimized Prediction Method with Alignment) is an improvement of SOPM method. The improvent takes place in the fact that SOPMA takes into

account information from an alignment of sequences belonging to the same family. If there are no homologous sequences the SOPMA prediction is the SOPM one.

CLUSTAL W

ClustalW is a widely used system for aligning any number of homologous nucleotide or protein sequences. For multi-sequence alignments, ClustalW uses progressive alignment methods. In these, the most similar sequences, that is, those with the best alignment score are aligned first. Then progressively more distant groups of sequences are aligned until a global alignment is obtained. This heuristic approach is necessary because finding the global optimal solution is prohibitive in both memory and time requirements. ClustalW performs very well in practice. The algorithm starts by computing a rough distance matrix between each pair of sequences based on pairwise sequence alignment scores. These scores are computed using the pairwise alignment parameters for DNA and protein sequences

MEGA 4

Reconstruction of the evolutionary history of genes and species is currently one of the most important subjects in molecular evolution. If reliable phylogenics are produced, they will shed light on the sequence of evolutionary events that generated the present day diversity of genes and species and help us to understand the mechanisms of evolution as well as the history of organisms. Phylogenetic relationships of genes of organisms are usually presented in treelike form with a root, which is called a rooted tree. It is also possible to draw a tree without a root, which is called unrooted tree. The branching pattern of a tree is called a topology. MEGA constructs Phylogenetic tree in Distance Method, UPGMA method, Maximum Parsimony method and Likelihood method. It constructs the phylogenetic tree, with bootstrap value. For constructing the phylogenetic tree, the protein sequences are needed to be aligned and converted into .meg file and .aln file. From the aligned results, one can infer the similarities and variations from the multicolour output. A tree would be constructd using aligned sequence file.

III. RESULTS AND DISCUSSION

In furtherance to gain an intuition into the comparative analysis of lymphoid tumour protein was conducted in seven protein sequence. The protein sequences were retrieved from NCBI database for BCL-2, BCL-6, BCL-10, BCL-11, BCL-W, BCL-XL and CD. The PROTOPARAM tool was used to find out the physical and chemical parameters of the seven proteins taken for present investigation. The analysis reveals that the protein BCL-6 has more number of amino acids (9582) and the protein BCL-11 has less number of amino acids (894). The numbers of amino acids present in a proteins sequence determine the molecular weight of proteins. Hence the protein BCL-6 is heavier than the other proteins (1040860.0 kDa).
A protein's Isoelectric Point or pI is the pH at which the protein has an equal number of positive and negative charges. The pI of BCL-10, BCL-10 and BCL-W is in the range of 6.10 – 6.37. The pI of the protein BCL-XL and CD-20 is somewhat in the range of 5.09 – 5.19. The protein BCL-6 which has more number of amino acids has high pI of 8.36. The instability index showed that the protein BCL-W is the stable protein which has the value of lesser than 40 (33.17). All the other proteins are unstable (Table 1).

The secondary structure prediction of the seven proteins was done using SOPMA. The structure of a protein is decided by the percentage of Alpha Helix, Beta sheet and Coil region. The BCL-2 has more alpha helix region. The range of alpha helix percentage varies from 3.91% to 62.50%. The secondary structure of BCL-2 of Cat, Human and Mouse is somewhat similar and Rat and Horse share somewhat similar structure. The protein BCL-6 has more coil region. The range of coil region percentage varies from 60.40% - 84. 23%. Monkey, Human and Chimpanzee has the same proteins structure. The structure found in Mouse is somewhat similar to the above said three species. The protein BCL-10 has more percentage of coil that alpha helix and beta sheets. The percentage of coil region varies from 44.35% - 51.62%. Chimpanzee and Human share the same structure. Though the sequence length of Mouse, Chimpanzee, Pig, Human and Rat are same (233), the structure among the 5 said species varies. The difference in the structure might impact in the difference in functions of the proteins. More research needs to be done to find out the differences in the function. The protein BCl-11 in Human has more coil region (60.51%). The protein

BCL-W has more alpha helix region. The range in 6 species varies from to 30.97%to 65.80%. The structure of proteins BCL-W found in Dog and Horse is exactly same. The sequence length of Dog, Pig, Horse and Mouse is same (193 amino acids). But except Horse, Dog and Mouse does not share the same structure and the structure slightly varies. More research need to be done to find out the functional similarity based on the structure similarity. The protein BCL-XL has more alpha helix region. The range in the species taken for the study varies from 23.74% to 54.08%. The results reveal that, Cat and Pig has same sequence length (233 amino acids). But the structure varies which might result in the difference in function. The protein CD-20 has both alpha helix and coil region in equal percentage. The range of alpha helix varies from 20.90% to 47.74% and coil region range varies from 30.45% to 51.20%. Horse, Human and Pig shows a similar structure (Table 2).

Multiple sequence alignment of the seven proteins sequence was taken for present study using Clustal W. The multiple sequence Alignment BCL-2 proteins shows that sheep and rabbit posses the same conserved substitutions. This protein might undergo mutation during the evolution. The multiple sequences Alignment of BCL-6 protein shows that Human, Chimpanzee, Rhesus Monkey, Mouse and Horse have the same conserved substitutions. The amino acids Leucine (L) and proline (P) is present as conserved residue in same position in all the species taken for the study. This finding would help in tracking the function of a protein. The multiple sequence alignment of BCL-10 shows that except Rhesus Monkey, all the species possess same conserved residues, conserved and semi conserved substitutions. As the sequence length of Rhesus Monkey is longer than the other species, the Clustal-W tool was not able to align it properly. The finding envisages that the function of BCL-10 proteins might be similar as they share common conserved residue, conserved and semi conserved substitutions. The multiple sequence alignment of BCL-W proteins shows the amino acids Glycine (G), Leucine (L), proline (P) and Glutamic acid (E) are present as a conserved residue in all the species taken for the study. Dog, Horse, Pig and Mouse share common conserved substitutions.

Meziane *et al*. [11] reported that the venous insufficiency and lymphatic stasis have already been incriminated in the protein is of this type of lymphoid tumors the prior injury and resulting immune dysregulation at the burn site may have also contributed to the development of this neoplasia. Identification of BCL6 target protein indicate a critical role for BCL6 in facilitating a state of physiological genomic instability required for GC B-cells to undergo affinity maturation and suggest its contribution to several additional cellular functions. The discovery of several layers of counter-regulatory mechanisms reveals how B-cells can control and fine-tune the potentially Lymphoidgenic actions of BCL6. From the biochemical standpoint, BCL6 can regulate distinct biological pathways through different cofactors. This observation explains how the biological actions of BCL6 can be physiologically controlled through separate mechanisms and affords the means for improved therapeutic targeting. The fact that patients with BCL6-dependent Lymphoid Tumors can be identified based on protein signatures suggest that therapeutic trials of BCL6 inhibitors could be personalized to these individuals. BCL6 plays a fundamental role in lymphoid proteinis and is an excellent therapeutic target for development of improved anti-lymphoid tumors therapeutic regimens [12]. BCL 10 phosphorylation isoforms has revealed a new mechanism controlling BCL10 nuclear translocation and an unexpected role for BCL 10 in the regulation of the actin cytoskeleton [13]. Jost *et al*. [14] opined that the development of Lymphoid tumors and leukemias is frequently caused by chromosomal translocations that deregulate cellular pathways of differentiation, proliferation or survival. The molecules that are involved in these aberrations provide rational targets for selective drug therapies. Recently, several disease specific translocations have been identified in human MALT Lymphoid tumors. BCL11A coamplified with Rel in B- NHL cases and HD Lymphoid Tumors cell lines with gains and amplifications of 2p13, suggesting that BCL 11A may be involved in Lymphoid Tumors malignancies through either chromosomal translocation or amplification [15]. The anti-apoptotic BCL-w regulator, which is expressed in the developing and mature brain, not only promotes neuronal survival, but also neuronal differentiation. However, its transcriptional regulation remains to be elucidated due to a lack of knowledge of the Bcl-w promoter, which is highly conserved between the human, mouse and rat species. Using a series of 5' and 3' deletions, the TATA-less minimal Bcl-w promoter and showed that it is under a combinational regulation with the neurogenic bHLH transcription factor Neuro D6 mediating its activation, validating previous finding of increased

expressionof the Bcl-w protein in stably transfected PC12- Neuro D6 cells. Upon stress, NeuroD6 promotes colocalization of Bcl-w with mitochondria and endoplasmic reticulum. Finally the first evidence of Bcl-w localizationin the growth cones of differentiating neuronal cells, suggestive of a potential synaptic neuroprotective role [16].

Bcl-xL and Bcl-2 and evidence suggests that phosphorylation disables their anti-apoptotic activity. However, the responsible kinase has remained elusive. In this report evidence is presented that CDK1/cyclin B catalyzes mitotic arrest-induced Bcl-xL/Bcl-2 phosphorylation. Furthermore, that CDK1 transiently and incompletely phosphorylates these proteins dueing normal mitosis. When mitosis is prolonged in the absence of microtubule inhibition, Bcl-xl and Bcl-2 become highly phosphorylated. Transient overexpression of non-degradable Bcl-xl mutant but not by a phosphor-mimic Bcl-xl mutant, confirming Bcl-xL as a key target of pro-apoptotic CDK1 signaling. These findings suggest a model whereby a switch in the duration of CDK1 activation, from transient during mitosis to sustained during mitotic arrest, dramatically increases the extent of Bcl-xl/Bcl-2 phosphorylation, resulting in inactivation of their anti-apoptotic function. Thus, phosphorylation of anti-apoptotic Bcl-2 proteins acts as a sensor for CDK1 signal duration and as a functional link coupling mitotic arrest to apoptosis [17].
T-cell prolymphocytic leukemia (T-PLL) was successfully treated with fludarabine monophosphate. Peripheral blood examination showed anemia and leukocytosis with 29.5% abnormal lymphocytes. The bone marrow was infiltrated with 84.1% abnormal lymphocytes. The nucleolus was visible in some of these abnormal cells. These cells were positive for CD2, CD3, CD4, CD5, CD7, CD38, CD52 and negative for CD8, CD10, CD19, CD20, CD25, CD56. Based on these findings, it was diagnosed as having T-PLL. Therapy with oral cyclophosphamide (50mg/day) was started, but was discontinued becaue of agranulocytosis. When received intravenous fludarabine monophosphate (30mg/day) per day 1-5 every four to five weeks. The reticulocyte count increased gradually and became free from red cell transfusions [18].

These finding show that BCL-W might had undergone mutation in Pig, Dog, Horse and Mouse. More investigation need to be done to trace the actual mutation occurred among the species. From the multiple sequence alignment of BCL-XL proteins, it can be inferred with the amino acid proline (P) is present as a conserved residue in all the species taken for the study. Cattle, Pig, Cat and Dog have common conserved and semi conserved substitutions. The multiple sequence alignment of CD-20 shows that the amino acids Glutamic acid, Proline and Glycine are present as conserved residues in all the species taken for the study. Mouse, Rat, Cat and Rhesus monkey have common conserved and semi conserved substitutions. An interesting finding of multiple sequence alignment is that the amino acid proline is present in all the proteins of lymphoid tumor and in the species taken for the study. From this it could be inferred that the amino acid proline might be responsible in causing lymphoid tumour. More research needs to be done to target proline to find out effective and efficient drug to cure lymphoid tumor.

The tree of each protein was constructed with Bootstrap value, The Bootstrap values are calculated as a percentage of the number of times that the particular node appears when performing Bootstrapping analysis on constructing phylogenetic tree. E.g. If there are 1000 iterations of bootstrapping and a node appears in 950 of the constructed tree then it has a Bootstrap as 95%. If a node genetically appears in less than 95% of total number of analysis, then it is regarded as being less accurate. Lower values are to be regarded with even less confidence. The Bootstrap value is used to predict the reliability of the phylogenetic tree constructed. The phylogenetic tree of the BCL-2 shows that the species, Rabbit and sheep are closel related. This node is somewhat closely related with Cat. The phylogenetic tree of the BCL-6 shows that the species, Pig and Horse are closely related. Monkey and Human are closely related. This node is closely related with Mouse, Chimpanzee and Human, which is closely related with Monkey. The phylogenetic tree of the BCL-10 shows that the species, Mouse and Rat are closely related. This node is somewhat closely related with Cattle. The phylogenetic tree of the BCL-W shows that the species, Horse and Pig are closely related. This node is closely related with Mouse. The phylogenetic tree of the BCL-XL shows that the species, Cattle and Pig are closely related. Dog and Mouse are also closely related. The phylogenetic tree of the CD-20 shows that the species, Mouse and Monkey are closely related. Cattle and Rat are closely related. Horse and Dog are closely related. This node is somewhat closely related with Human. The

results of this research work could be utilized for further detailed investigations on the seven proteins involved in lymphoid tumor to formulate measures for the betterment of mankind.

IV. CONCLUSION

Lymphoid tumor is a cancer that begins in the lymphocytes of the immune system and present as a solid tumor of lymphoid cells. Lymphoid tumor is the most common form the hematological, malignancy or blood cancer in the developed world. Treatment of lymphoid tumor is often long and confusing dealing with side effects and tests might be difficult. Still research is going on lymphoid tumor to find out the effective drug or treatment to completely cure lymphoid tumor. This research work was undertaken to find out the common proteins involved in lymphoid tumor and to find the evolutionary relationship of different species having the proteins involved in lymphoid tumor.

V. REFERENCES

[1]. Roszkowski K. 2014. oxidative DNA damage - the possible use of Biomarkers as additional prognostic factors in oncology. Front biosci. (Landmark ED.) 19, 808-817,

[2]. Gorrini C., Harris I.S., and Mak T.W. 2013. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov. 12 (12): 931-947.

[3]. Jemal, A., Bray, F., Canter, M.M., Ferlay, J., Ward, E. and Forman, D. 2011. Global cancer statistics. CA cancer J clin. 61(2):134.

[4]. Benaglia, M. 2009. Recoverable and Recyclable Catalysts, John Wiley and Sons, Chichester.

[5]. Cushing, B.L., Kolesnichenko, V.L., Connor, C.J.O. 2004. Chem. Rev., 104, 3893.

[6]. Astruc, D. 2008. Nanoparticles and Catalysis;, Ed.; Wiley-VCH: Weinheim.

[7]. Vardiman, J.W., Thiele, J., Arber, D.A., Brunning, R.D., Borowitz, M.J., Porwit, A., Harris, N.L., Le Beau, M.M., Hellstrom-Lindberg, E., Tefferi, A. and Bloomfield, C.D. 2008. "The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes.". Blood. 114 (5): 937-51.

[8]. Adams, J.M., Cory, S. 2007. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 26(9):1324-1337.

[9]. His, E.D. and Yegappan, S. 2001. Lymphoma immunophenotyping: a new era in paraffin-section immunohistochemistry. Adv Anat Pathol. 8:218-39.

[10]. Bilalovic, N., Blystad, A.K. and Golouh, R. 2004. Expression of Bcl-6 and CD10 protein is associated with longer overall survival and time to treatment failure in follicular lymphoma. Am J Clin Pathol. 121:34-42.

[11]. Meziane, M., Hesse, S., Chetaille, B., Bien - aimee, A., Grob, J.J., Richard, M.A. 2009. Cutaneous Lrge B-cell Lymphoid occurring on a leg burn. Ann Dermatol Venerol., 136(11): 791-794

[12]. Weimin, C.I, Jose, M., and Ari Melnick. 2009. B-cell lymphoid 6 and the molecular pathoproteins of diffuse large B- CELL lymphoid. Curr opin Hematol., 15(4): 381-390.

[13]. Thome, M. and Weil, R. 2007. Post translational modifications regulate distnict functions of CARMA1 and BCL10. Trends immunol., 28(6) :281-288.

[14]. Jost, P., Peschel, C. and Ruland, J. 2006. The Bc110/Malt1 signaling pathway as a drug target in lymphoid. Curr Drug Targets., 7 (10): 1335-40

[15]. Satterwhite, E., Sonoki, T., Willis, T.G., Harder, L., Nowak, R., Arriola, E.L., Liu, H., Price, H.P., Gesk, S., Steinemnn, D., Schlegelberger, B., Oscier, D.G., Siebert, R., Tucker, P.W. and Dyer, M.J. 2011. The BCL11 protein family: involvement of BCL11A in lymphoid malignancies. Blood., 98 (12):3413-3420.

[16]. Uittenbogaaard, M., Baxter, K.K. and Chiaramello, A. 2009. Cloning and characterization of the 5UTR of the rat anti-apoptotic BCL- w protein. Biochem Biophys Res Commun., 389 (4): 657-662.

[17]. Terrano, D.T., Upreti, M. and Chambers, T.C. 2009. CDK1- mediate BCL-XL/BCL-2 phosphorylation acts as a functional link coupling mitotic arrest and apoptosis. Mol Cell. Biol.

[18]. Maeda, A., Iwai, K. and Ishibashi, T. 2009. Sucesssful treatment of T- Cell prolymphocytic leukemia (T-PLL) with fludarabine monophosphate. Rinsho Ketsueki. 50 (8): 658-662.