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Identification of Therapeutic Drug for Non-Hodgkin's Lymphoma Using Insilico Methods

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ABSTRACT: Cancer is a dangerous group of diseases. There are over 100 different types of cancer each affecting different parts of the body and almost every tissue in the body can get affected with either a benign tumour or malignant cancer.In this paper, we have worked with Non-Hodgkin's Lymphoma. For carrying out the experimental work, 15 sequences were taken and processed to find out their protein families. Through careful selection, 3 sequences, with the strongest possible affinity to drugs, were selected for analysis. The proteins were subjected to BLASTP for selecting templatesand for generation of a new protein structure, using the MODELLER software. The quality of the protein models was verified. Further, the proteins were docked against 3 known, approved drugs (Rituximab, Vincristine, Rasburicase), *Annonamuricata*, a rumoured medicinal plant. The computerized result showed that only one of the protein models was able to bind to the drugs, and that the medicinal plant had a better effect on the protein than the approved drugs. We could conclude from this paper that the new protein model generated could be useful for further studies on Non-Hodgkin's' Lymphoma, and that the plant could possibly be used as an alternative to chemotherapy in the future.

KEYWORDS: Cancer, Non-Hodgkins' lymphoma, Soursop, Modeller

I. INTRODUCTION

Non-Hodgkin's lymphoma is a large group of cancers of lymphocytes (white blood cells). They can occur at any age and are often marked by lymph nodes that are larger than normal, fever, and weight loss. They are mainly found in the lymph system. Adult non-Hodgkin lymphoma can begin in almost any part of the body and can occur in both adults and children. Treatment for children, however, is different than treatment for adults. Scientists are making a lot of progress in understanding how changes in DNA can cause normal lymphocytes to develop into lymphoma cells. Once this is understood, drugs may be developed that block this process.Progress in understanding DNA changes in lymphoma has already provided improved and highly sensitive tests for detecting this disease.Treatment of the cancer depends on different factors such as the specific type of lymphoma, its stage, one's age and overall health, etc. Treatment can include Chemotherapy, Bone marrow transplants, Targeted therapies, Lymphoma vaccines, etc. However, there are many possible complications, including autoimmune hemolyticanaemia, infection and side effects of chemotherapy drugs.In the present study,we have carried a study on this by taking 15 proteins from this disease, blasted in PDB for similar structures, found active sites and have tried to dock the validated structures with 3 drugs and 2 medicinal plants. The results of the same have been discussed in the section Results and discussion.

II. RELATED WORK

Berinstein*et al*., worked on Association of serum Rituximab (IDEC-C2B8) concentration andanti-tumor response in the treatment of recurrent low-grade orfollicular non-Hodgkin's lymphoma [1]. They concluded that Rituximab is therapeutically effective against B-cell lymphoma. Pearce *et al*., did a case-control study on Non-Hodgkin's lymphoma and exposure to phenoxyherbicides, chlorophenols, fencing work, and meat works employment in the year [2]. They concluded that the exposure to phenoxyherbicides and chlorophenols, along with fencing meat works did have a negative effect and caused increase risk of developing non-Hodgkin's lymphoma. Zahm and Blair worked on Pesticides and Non-Hodgkin's Lymphoma [3]. Their paper reviewed the role of pesticides in this increase. They concluded that, since the use of pesticides, particularly phenoxyherbicides, had increased dramatically before and during the time period in which the incidence of NHL has increased, they could have contributed to the rising incidence of NHL. O'Connor *et al*., worked on Phase II clinical experience with Bortezomib in patients with indolent Non-Hodgkin's

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Lymphoma and Mantle Cell Lymphoma [4]. Their data suggested that bortezomib was well tolerated and had significant single-agent activity in patients with certain subtypes of NHL. Guglielmi*et al*., worked on autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma [5]. Their studies showed that the overall rate of response to conventional chemotherapy was 58 percent; among patients with relapses after chemotherapy, the response rate was 64percent, and among those with relapses during chemotherapy,the response rate was 21 percent. They concluded that, as compared with conventional chemotherapy, treatment with high-dose chemotherapy and autologous bone marrow transplantation increased event-free and overall survival in patients with chemotherapy-on-Hodgkin's lymphoma in relapse. Purdue *et al*., worked on subclinical immune system function and its influence on lymphomagenesis [6]. They conducted a nested case–control study within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial to investigate whether circulating levels of cytokines and other immune markers are associated with future risk of NHL. They concluded that their findings for sTNF-R1 and sCD27, could be possible markers for inflammatory and B-cell stimulatory states, respectively, supported a role for subclinical inflammation and chronic B-cell stimulation in lymphomagenesis. Rousseaux*et al*., worked on identifying a novel BET bromodomain inhibitorsensitive, gene regulatory circuit that could control Rituximab response and tumour growth in aggressive lymphoid cancers [7].They discovered a gene regulatory circuit involving the nuclear factor CYCLON, which characterizes aggressive disease and resistance to the anti-CD20 monoclonal antibody, Rituximab, in high-risk B-cell lymphoma. They concluded that CYCLON was a new MYC cooperating factor that autonomously drives aggressive tumour growth and Rituximab resistance in lymphoma, and that its resistance mechanism would eventually provide a new combination therapy rationale for high-risk lymphoma. Demurtas*et al*., did a retrospective analysis of 1,792 solid tissues suggestive of lymphoma, submitted over a 12-year period, was carried out and flow cytometry (FC) results were compared with histologic findings [8]. Results showed that FC routinely performed on tissue samples suspected of lymphomas was a fundamental adjunct to morphology in the diagnosis of NHL. They concluded that this could enhance the performance of the histologic evaluation so as to achieve the final diagnosis.

III. MATERIALS AND METHODS

A. *Data Collection*

Around 14 protein sequencesfor Non-Hodgkin's Lymphoma were collected from NCBI Protein database. The sequences were collected in FASTA format, and all were found to be confirmed sequences through wet-lab tests, i.e.,none were computationally predicted.

B. *Identifying the Protein Families*

The protein families of the sequences were searched by using Pfam database, found on http://pfam.xfam.org/search. The Pfam database is a large collection of protein families, each represented by multiple sequence alignments and hidden Markov models (HMMs). There are two components to Pfam: Pfam-A, which consists of high quality, manually curated families, and Pfam-B, consisting of computationally predicted supplements using the ADDA database.

C. *Performing BLAST for Proteins*

The protein sequences were checked for their identities by running a BLASTP against the NCBI database, using the PDBdatabasecategory(http://blast.ncbi.nlm.nih.gov/Blast.cgi). The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. Standard protein-protein BLAST (blastp) is used for both identifying a query amino acid sequence and for finding similar sequences in protein databases.

D. *Generating new Protein Using MODELLER*

During BLAST, the results which showed less than 60% Identity and Query cover, related to the disease/species, were selected as 'templates'. The protein templates for each protein were taken, and, using MODELLER, generated a new protein structure.MODELLER is used for homology or comparative modeling of protein three-dimensional structures. The user provides an alignment of a sequence to be modeled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms.

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E. *Verifying the Protein Quality*

The new proteins generated by Modeller were validated by plotting the amino acids, using the Ramachandran plot from the RAMPAGE site (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php). A Ramachandran Plot is a way to visualize dihedral angles ψ against φ of amino acid residues in protein structure. It shows the phi-psi torsion angles for all residues in the structure (except those at the chain termini). Ideally, one would hope to have over 90% of the residues in these "core" regions. The percentage of residues in the "core" regions is one of the better guides to stereochemical quality.

F. *Energy Minimization of the Proteins*

The selected proteins were subjected to Energy Minimization using Swiss-PDB viewer application that provides a user friendly interface, capable of analyzing several proteins at the same time. Swiss-PDB viewer can also read electron density maps, and provides various tools to build into the density. In addition, various modeling tools are integrated and residues can be mutated.

G. *Docking of the Proteins*

The validated protein pdb files were docked against the drugs and plants toprovide the least binding energy, using ArgusLab (http://www.arguslab.com/arguslab.com/ArgusLab.html). It is a program to build graphic representations of molecular models and allows you to build surfaces, calculate energy, optimize geometry, and perform Gaussian calculations, plot molecules and everything we need to customize our molecular model, including docking. The drug which would provide the least binding energy would be the most suitable therapeutic drug for the given malady. The protein and the alkaloid were then subjected to the Lipinski rule, through http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp.

IV. RESULTS AND DISCUSSION

The 14 protein sequences were analyzed through Pfam.The protein families were found to have a mix of Pfam A and B groups. The most common protein families found were a)TNFR c6 - TNFR/NGFR cysteine-rich region, b) Lectin-C - Lectin C-type domain, c) 5-FTHF - 5-formyltetrahydrofolate cyclo-ligase family. By selection, the sequences with Lectin-C families were chosen, i.e. 3 sequences were chosen.

The BLASTP gave good results against the PDB database. In order to generate a protein model, for each protein, the results which had Identities lower than 50% were taken as 'templates'. Seven templates for each protein were selected to generate a protein model.For each protein, seven template PDB files were taken. These templates were then entered, using Python code, and then run, using MODELLER, to generate seven new models for each protein, using the seven templates.For each of the three proteins, the seven protein models generated were verified using Ramachandran plot (RAMPAGE). The best of the seven models for each protein was selected based on its residues in favored regions. The model with the highest percentage was selected for docking. In total, three models for the three proteins were selected. The energy minimization was then done for each of the generated protein models. Slight differences were found before and after the minimization.

On docking with the drugs and plant, it was found that only one of the protein models, i.e. Test-3, had a good affinity towards the ligands, showing that this protein model could be used for further studies with respect to Non-Hodgkin's Lymphoma. It was also found that, although the drugs had a good effect on the proteins, with low binding energy, the plant alkaloid taken from *Annonamuricata*, was found to have a better effect on the proteins, with a lower binding energy. Running the Lipinski rule for the Soursop alkaloid also gave good results, as it was positive for all the Lipinski rules. This shows that Soursop could have future use as an anti-cancer drug.

V. CONCLUSION AND FUTURE WORK

From this paper, we could conclude that the new protein models generated could have possible use in analysis of Non-Hodgkin's Lymphoma and similarly related diseases.There is also a possibility for future use of *Annonamuricata* as a replacement for chemotherapy, or as an alternative medicine. In our future workwe would include applying

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Schrodinger software to validate the protein model and the alkaloid of *Annonamuricata*, and to confirm possible use of *Annonamuricata*as a drug for treatment of Non-Hodgkin's Lymphoma.

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