Identification of the motifs of beta-turns and mutated amino acids studies on BCR (Breakpoint cluster region) protein using Insilico techniques

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ABSTRACT

Recent investigations have rapidly added crucial new insights into the complex functions of the normal BCR gene and of the BCR-ABL chimaera. They are yielding potential therapeutic breakthroughs in the treatment of Philadelphia (Ph) chromosome-positive leukemias. The objective of the present in silico research investigation is to find out whether the functional part (beta-turns) is present in the mutated amino acids of BCR (Breakpoint cluster region) protein. Two significant steps are involved in this study. First, we performed protein sequence modeling of BCR using automated protein modeling servers and the 3D structure was visualized using molecular visualization software and tools. In the second step, the function domains and motifs regions of BCR gene-coded protein is predicted using “PDBsum generate” tool in order to show where exactly the beta-turns lie on the clinically-proven mutated amino acids of BCR protein. The results of our investigation can be used as potential drug binding sites in the field of drug docking studies. It can act as a potential therapeutic agent for Chronic Myeloid Leukemia (CML) type of Leukemia.

INTRODUCTION

Chromosomal aberrations related to BCR has been found in patients affected by chronic myeloid leukemia (Translocation t(9;22)(q34;q11) with ABL1). The translocation results in the production of BCR-ABL which is also observed in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (Chissoe et al., 1995; Hariharan and Adams, 1987). The structural organization of the BCR gene which contains 23 exons present on chromosome 22 has been determined (Hariharan and Adams, 1987). The first exon comprises of a unique serine/threonine kinase activity and at least 2 SH2 binding sites. The fact that the BCR gene is directed with its 5-prime end toward the centromere of chromosome 22 has been demonstrated (Stam et al., 1987). BCR gene is composed of 23 BCR exons with putative alternative BCR first and second exons (Chissoe et al., 1995). About 10% of patients with acute lymphocytic leukemia (ALL) have the translocation t(9;22)(q34;q11) indistinguishable from that of CML. It has however, been found in 3 out of 5 such cases of ALL that the bcr region was not involved and that the 22q11 chromosome breakpoint was proximal (5-prime) to the bcr region (Erikson et al., 1986). 2 Furthermore, the bcr and c-abl transcripts were normal sized in an ALL line carrying the t(9;22) translocation. The
breakpoints of the t(9;22) CML, the t(9;22) of acute lymphocytic leukemia, and the t(8;22) of Burkitt lymphoma fall into 22q11 and are cytologically indistinguishable. By chromosomal in situ hybridization, however, they can be distinguished (Emanuel et al., 1984; Griffin et al., 1986). To this experience, observations on t(11;22), both constitutional and tumor-related were added (Griffin et al., 1986). To block BCR/ABL function, a unique tyrosine phosphatase was created by fusing the catalytic domain of SHP1 to the ABL binding domain of RIN1, an established binding partner and substrate for c-ABL and BCR/ABL (Lim et al., 2000).

**METHODOLOGY**

The FASTA format sequence of BCR (Breakpoint Cluster Region) was retrieved from Uniprot (https://www.uniprot.org/uniprot/P11274) database and submitted to CPH 3.0 model server (http://www.cbs.dtu.dk/services/CPHmodels/) in order to perform protein modeling and 3D structure visualization. The modeled 3D structure was viewed and validated by Discovery Studio software. We also studied the location of beta-turns in the mutated amino acids (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) of the BCR protein structure. Finally, we predicted all the results shown in 3D form using visualization software.

**RESULTS AND DISCUSSION**

In this research, we focused on the BCR protein (Uniprot ID: P11274) target, the length of the nucleotide sequence is (3816 nt) and the protein length is (1271 aa) which is present in (22) chromosome.

The FASTA format of the protein sequence is given in Figure 1. The 3D structure of the protein target, BCR in Figure 2 is shown in secondary structure view model. Here, we used advanced molecular visualization software called discovery studio software. The picture displays the model of amino acids using CPH 3.0 model server (Lund et al., 2002; Nielsen et al., 2010) which shows the intramolecular 3D effects of the structural domains of BCR protein.
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Figure 4: 3D Structure of BCR Protein – Discovery studio software

Figure 5: 3D Beta turns in human BCR protein - PDBsum Generate

Figure 6: 3D Structure of BCR Protein (Beta-turns) – Discovery studio software

Figure 7: 3D Structure of BCR Protein (Beta-turns) – Discovery studio software

white-hydrogen of BCR protein.

In the above picture, red colour indicates helix and green colour -turns and white colour -coiled region of BCR protein (Figure 3).

In this in silico research investigation, we focused on where the 3D structural region of the mutated amino acids lies on the structural domains and motifs. We used Uniprot database which has clinically proven references that the amino acids (THR: 1127, ALA: 1149, GLU: 1161, LYS: 1189, ALA: 1204) (Figure 4) are directly involved in the BCR gene coded protein (Greenman et al., 2007).

In Figure 4, Red colour indicates helix and green colour –turns and white colour – coiled region with respective amino acids labels and amino acids position numbers of BCR protein.

Similarly, our results predicted the 3D structure of the beta-turns of the amino acids of BCR protein which is shown in Figure 5.

The Figure 5 shows the pdbsum generate tool results, total amount of beta turns present in BCR protein sequence.

The mutated amino acids positions are follows : (THR : 1127, ALA : 1149, LYS : 1187, VAL : 1189, ALA : 1204) whereas the predicted beta turns amino acids are (PHE: 6 1052, MET:1083, VAL:1086, ILE:1088, TYR:1189, ARG:1190, VAL:1114, MET:1115, MET:1116, GLU:1118, PRO:1136, LEU:1139, ASP:1142, GLU:1143) (Table 1).

The above table represents the positions of the predicted beta-tuns of amino acids and the positions of the clinically proven mutated amino acids of BCR protein.

The above picture represents the secondary structure model with predicted beta-turns of amino acids (Space fill model view) with respective positions (Figure 6).

The above picture represents the secondary structure model of the predicted amino acid and clinically proven mutated amino acid Lys 1187 (Space fill model view) with respective positions.

Interestingly, it has been found out in our research that the position of the predicted beta-turns of the amino acids lie in the same position as the clinically proven mutated amino acids. This has also been explained, with the help of molecular visualization tools such as Discovery Studio Software in Figure 7. These amino acids play a vital role in the drug binding sites which may act as potential target regions in BCR protein.
Table 1: Molecular protein mechanics profiling – BCR

| S. No. | Predicted positions of β turns of aa of BCR protein | Clinically proven positions of mutated aa of BCR protein |
|--------|-----------------------------------------------------|--------------------------------------------------------|
| 1      | PHE:1052                                            | THR:1127                                               |
| 2      | MET:1083                                            | ALA:1149                                               |
| 3      | VAL:1086                                            | LYS:1187                                               |
| 4      | ILE:1088                                            | VAL:1189                                               |
| 5      | TYR:1089                                            | ALA:1204                                               |
| 6      | ARG:1090                                            |                                                        |
| 7      | VAL:1114                                            |                                                        |
| 8      | MET:1115                                            |                                                        |
| 9      | MET:1116                                            |                                                        |
| 10     | GLU:1118                                            |                                                        |
| 11     | PRO:1136                                            |                                                        |
| 12     | LEU:1139                                            |                                                        |
| 13     | ASP:1142                                            |                                                        |
| 14     | GLU:1142                                            |                                                        |

CONCLUSION

In conclusion, the overall results of this in silico study proved that the identified structural motifs (beta-turns) lie within the same position range as the mutated amino acids of BCR protein. We also elucidated this with the help of 3D structure using advanced visualization techniques. The results of our study would act as novel target sites for CML type of leukemia. Our study can be extended in future to structure-based drug designing and docking on BCR protein. The novel identified drug target site would play a vital role in Cancer-informatics and Clinical Pharmacology.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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