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Bioinformatics Profiling of Missense Mutations

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***Abstract*—**The ability to distinguish missense nucleotide substitutions that contribute to harmful effect from those that do not is a difficult problem usually accomplished through functional in vivo analyses. In this study, instead current biochemical methods, the effects of missense mutations upon protein structure and function were assayed by means of computational methods and information from the databases. For this order, the effects of new missense mutations in exon 5 of PTEN gene upon protein structure and function were examined. The gene coding for PTEN was identified and localized on chromosome region 10q23.3 as the tumor suppressor gene. The utilization of these methods were shown that c.319G>A and c.341T>G missense mutations that were recognized in patients with breast cancer and Cowden disease, could be pathogenic. This method could be use for analysis of missense mutation in others genes.

***Keywords*—**Bioinformatics, missense mutations, PTEN tumor suppressor gene.

I. Introduction

THE word of mutation was primarily used by De Vries to describe a method for genesis of new species, though it is quite different than the current definition. Many different types of mutations are in the human genome and they are classified in two main group including major gene rearrangements and point mutations. Point mutations are single substitutions of specific bases in DNA including adenine, guanine, cytosine, and thymine. Alterations in the DNA sequence can result in an alteration of the protein sequence, expression or function. Point mutations within a gene that results a substitution of one amino acid to another in a protein were called missense mutations. The effects of missense mutations range from early lethality in fetal development to no observable phenotypic changes [1].

The effects of mutations on a proteins structures and functions have until recently only been assigned by laborious biochemical characterization of the mutant proteins. In these days computational methods and wide range of information from the databases containing information on DNA and protein sequences and on protein structure and function are exploited in order to the assessment of the effects of mutation on the protein structure and function [2]. In this study computational methods were used to examine the effects of new missense mutations in exon 5 of PTEN tumor suppressor gene upon protein structure and function.

The gene coding for PTEN (Phosphatase and Tensin homolog deleted on chromosome TEN), also called MMAC1 (Mutated in Multiple Advanced Cancers) was identified and localized to chromosome region 10q23.3 as the tumour suppressor gene [3,4] responsible for susceptibility to Cowden (CD) [5], Lhemitte-Duclos (LDD) and to Bannayan-Zonana syndromes (BZS) [6].

PTEN contains 9 exons and encodes a 403 amino acid lipid phosphatase that dephosphorylates D3 of phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3) in opposition to phosphatidylinositol 3 kinase (PI3K) activity. PTEN is a member of the large PTP (protein tyrosine phosphatase) family [7].

The structure of PTEN consists of an N terminal phosphatase domain followed by an associated C terminal C2 domain [8]. The phosphatase domain contains the active site which carries out the enzymatic function of the protein. C-terminal tail contains a cluster of serine and threonine residues (PEST sequence) that become phosphorylated in many cells, and a binding site for a group of PDZ domain containing proteins. The C2 domain allows PTEN bind to the phospholipid membrane and dephosphorylation of PI3P [9].

The purpose of this study was assessment of the pathogenicity of amino acids substitution in phosphatase domain of PTEN protein by computational methods and information from the databases.

II. Materials and Methods

The studied material comprised of 2 new missense mutation and 34 single nucleotide polymorphism in coding and noncoding regions of PTEN gene. For traditional sequencing, PCR products were amplified from genomic DNA extracted from somatic cell. Sequence reactions were done using conventional Sanger sequencing methods for sense directions. Traces were analyzed using Sequencher (Gene Codes, Ann Arbor, MI).

At the first step of study, in a order to establish the novelty of mutations the databases NIEHS (National institute of environmental health science) and COSMIC (Catalogue of Somatic Mutations in Cancer) were searched [10, 11].

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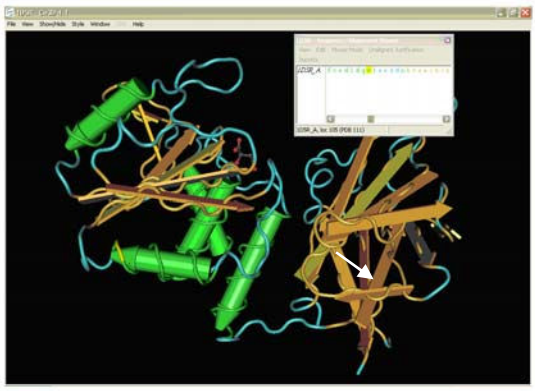
An amino acid substitution is predicted to affect function or structure of the PTEN protein if one of the following conditions is apply in its case:
(1) The substitution is located in a site defined in the SWISS-PROT database as binding site, active site, and site involved in a disulphide bond etc (Primary accession number of PTEN protein on SWISS-PROT database: P60484). Missense mutations are known to cause Mendelian disease have been annotated in Swiss-Prot and studied for their deleterious effects on protein function [12]. The position of amino acid replacement in experimentally known 3D structure human PTEN protein in PDB was assessed.
(2) The position of amino acid substitution is conserved in the family of homologous proteins [13, 14]. The BLASTP was performed on human PTEN protein with homologous proteins from Xenopus and Drosophila. BLASTP was run with an E-value cutoff of 0.01. Modification of phrases regard the functional annotation such as "putative" and "-related" was ignored. An annotation including EC number was considered unambiguous [15]. The evolutionarily conserved amino acids in homolog proteins between species imply an important functional role for these residues in protein.
(3) The substitution destroys the hydrophobic core of the protein.
We also evaluated the impact of the observed new missense mutations on the secondary structure of the PTEN protein using MacVector software version 10.2.
(4) The substitution involves a proline and glycine residues in the α-helix.

III. Results

New Mutations in PTEN gene were included missense mutations (G→A) at nucleotide 1123 resulting in an aspartic acid (D) to asparagine (N) change at codon 107 (D107N) and (T→G) at nucleotide 1135 resulting in a tryptophan (W) to glycine (G) change at codon 111 (W111G) [5, 16]. The positions of mutations in genomic DNA and its corresponding protein were assigned by Sequence Extractor [17].

Asp-107 of the PTEN α3 helix is in position as the corresponding PTP1B Asp-196, which serves as a general acid to stabilize a turn at the α3 helix through backbone hydrogen bonds. Similar to the PTPs, missense mutation of Asp-107 in PTEN results in disruption of an interdomain hydrogen bond network. Trp-111 of the PTEN α3 helix in phosphatase domain is in position as the corresponding PTP1B Trp-204, which is hydrophobic and tends to orient towards the interior of the folded protein molecule (Fig. 1) [6].

(a)



(b)

Fig. 1 The positions of Asp-107 and Trp-111 in three dimensional structure of the PTEN protein were distinguished by Cn3D software. A) Asp-107 in pα3 helix is marked with arrow. B) Trp-111 in pα3 helix is marked with arrow

The alignment of the human PTEN protein with homologous proteins from Xenopus and Drosophila indicated that Asp-107 residue is highly evolutionarily conserved, implying an important functional role for D107 residue in the PTEN protein. In case of Trp-111 The alignment indicated that Trp-111 residue is conserved in Drosophila but in Xenopus is replaced by leucine (L) that like tryptophan is hydrophobic amino acid (Fig. 2).

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                                                107       111
Human       AQYPFEDHNPPQLELIKPFCEDLDQWLSEDDNHVAAIHCK
Xenopus     AQYPFEDHNPPQLELIKPFCEDLDQLLSEDDN-VAAIHCK
Drosophila  AVYPFDDHNPPTIELIQRFCSDVDMWLKEDSSNVVAVHCK
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Fig. 2 The alignment the phosphatase domain of human PTEN protein with homologous proteins from Xenopus and Drosophila obtained with the program BLASTP. The similar sequences are highlighted in yellow

Impact of the observed new missense mutation on the secondary structure of the PTEN protein was analyzed with MacVector software package (Genetics Computer Group, Madison, WI) version 10.2. The protein analysis suggested

that these mutations resulted in a detectable change of the surface accessibility of the protein in a region surrounding the altered residues.

c.341T>G mutation changes tryptophan to glycine at codon 111. Tryptophan contain large rigid aromatic group on the side chain and is one of the biggest amino acids. Tryptophan is hydrophobic and tends to orient towards the interior of the folded protein molecule. Glycine is the smallest amino acid, rotates easily, and adds flexibility to the protein chain. As too much flexibility is usually not desired.

34 number of missense polymorphism were collected from dbSNP with an overall minor allele frequency (MAF) >20%. None of aforesaid conditions was not apply in these cases.

IV. Discussion

In this study the effects of new missense mutations in exon 5 of PTEN tumor suppressor gene upon protein structure and function were studied by means of computational methods and information from the databases. After structural and functional analysis of mutations the significance of missense mutations were proved.

PTEN has central role as a negative regulator of the ubiquitous PI3K/AKT signaling pathway. A large proportion of mutations in PTEN have been mapped to the exon 5, region encoding the phosphatase domain (codon 90 to 142) [18].

The ideal method to assess the significance of missense mutations would include functional analysis of the translated protein. In this research, we take advantage of bioinformatics tools and information in order to analysis of single nucleotide substitution in PTEN gene. Four criterions were chosen for this purpose. If one of criterions is applying in one case, we conclude that nucleotide substitution has harmful effects on protein structure and function.

Asp-107 and Trp-111 serves to stabilize a turn at the α3 helix through backbone hydrogen bonds. These amino acids are in position as the corresponding to Asp-107 and Trp-204 in protein tyrosine phosphatases 1B (PTP1B) protein which is hydrophobic and tends to orient towards the interior of the folded protein molecule. Missense mutation in Asp-107 and Trp-204 of the PTPs results in disruption of an interdomain hydrogen bond network. The c.341T>G mutation was observed in evolutionarily conserved position.

The protein analysis with MacVector software package was suggested that these mutations resulted in a detectable change of the surface accessibility of the protein in a region surrounding the altered residues.

Experimental examination of missense mutations in codons 107 and 111 revealed that these PTEN missense mutations cause total loss of PTEN function and that all of them had no effect on the membrane binding activity of protein [19].

Several computational methods, including Sorting Intolerant from Tolerant [20], large-scale annotation of coding non synonymous SNPs [21], statistical geometry methods [22], and support vector machine methods [23] have been developed to identify deleterious mutations, however, their efficient in clinical utilization remains to be determined.

In clinical laboratories of genetics many new mutations in different genes are diagnosed daily. However, any standard protocol or special software for evaluation the effects of new mutations upon proteins structure and function has been not produced. In present manuscript this vital question is brought forth for discussion and on the temporary basis, a way out of the difficulty is presented. In order to distinguish polymorphisms from harmful missense mutations, this method does remarkably well in a number of different experiments.

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