Consideration of pathogenicity of nsSNVs in *CDKN2A* gene, as a new tumor marker for leukemia, using bioinformatics methods

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Abstract

CDKN2A as a tumor suppressor gene (TSG) encodes p14 and p16 that they are tumor suppressor proteins and cell cycle regulators. Sequence deletions or promoter hypermethylation lead to downregulation of these proteins. Also, point mutations can be caused malfunction or dysfunction of proteins. The aim of this study is the identification of deleterious non-synonymous single nucleotide variants (nsSNVs) for planning targeted experimental methods. We study three nsSNVs including rs104894095, rs786204195 and rs104894098 from NCBI/dbSNP databank. Then, these nsSNVs are considered by bioinformatics tools with different approaches such as SIFT, PolyPhen-2, I-Mutant2.0, P-Mut, ExPASy resource portal, PEPTIDE 2.0 web server. Also, we study hydrogen bonds and atom distances in these mutations by PyMOL software.

Methods

Data collection:

We identify and retrieve three nsSNVs in the *CDKN2A* gene from NCBI database. Also, we retrieve protein structure from PDB databank.

Predicting the effects of mutations:

We use different web-based bioinformatics tools for prediction of deleterious or benign effects of amino acid substitution like SIFT (Sorting Intolerant From Tolerant, score<0.05: deleterious), PROVEAN (Protein Variation Effect Analyzer, Score<-2.5: deleterious), PolyPhen-2 (Polymorphism Phenotyping v2) and P-Mut (score>0.5: Disease). Also, protein stability is estimated using I-Mutant2.0 (DDG<0: Decreased stability and DDG>0: Increased stability). Hydrophobicity alterations in substituted amino acid position are considered by ExPASy resource portal and PEPTIDE-2 tool. Also, we study mutant protein structure using PyMOL software and via comparing to wild type protein structure.

Results & Discussion

TABLE 1: EFFECT OF MUTATIONS

nsSNV ID	AA. change	SIFT	PROVEAN	PolyPhen-2	P-Mut
rs786204195	p.P48S	T 0.29	D -3.64	PD 0.973	Disease 0.7417
rs104894095	p.M53I	T 0.59	D -3.68	PD 0.966	Disease 0.5893
rs104894098	p.V126D	APF* 0.00	D -6.22	PD 1.000	Disease 0.8947

*There is low confidence in this prediction. Notice that AA: Amino acid, T: Tolerated, APF: Affected Protein Function, D: Deleterious, PD: Probably Damaging.

TABLE 2: EFFECT OF MUTATIONS

nsSNV ID	AA. change	PEPTIDE-2 prediction hydrophobicity index*	Change the nature of the amino acid	Amount of hydrophobicity change evaluated by ExPASy
rs786204195	p.P48S	Hydrophobic: 49.36% Neutral: 22.44%	Hydrophobic to Neutral	0.010
rs104894095	p.M53I	Not change	Very Hydrophobic to Very Hydrophobic	0.032
rs104894098	p.V126D	Hydrophobic: 49.36% Acidic: 14.74%	Very Hydrophobic to Hydrophilic	-0.095

*Index for wild type: Hydrophobicity: 50%, Acidic: 14.1%, Basic: 14.1%, Neutral: 21.79.

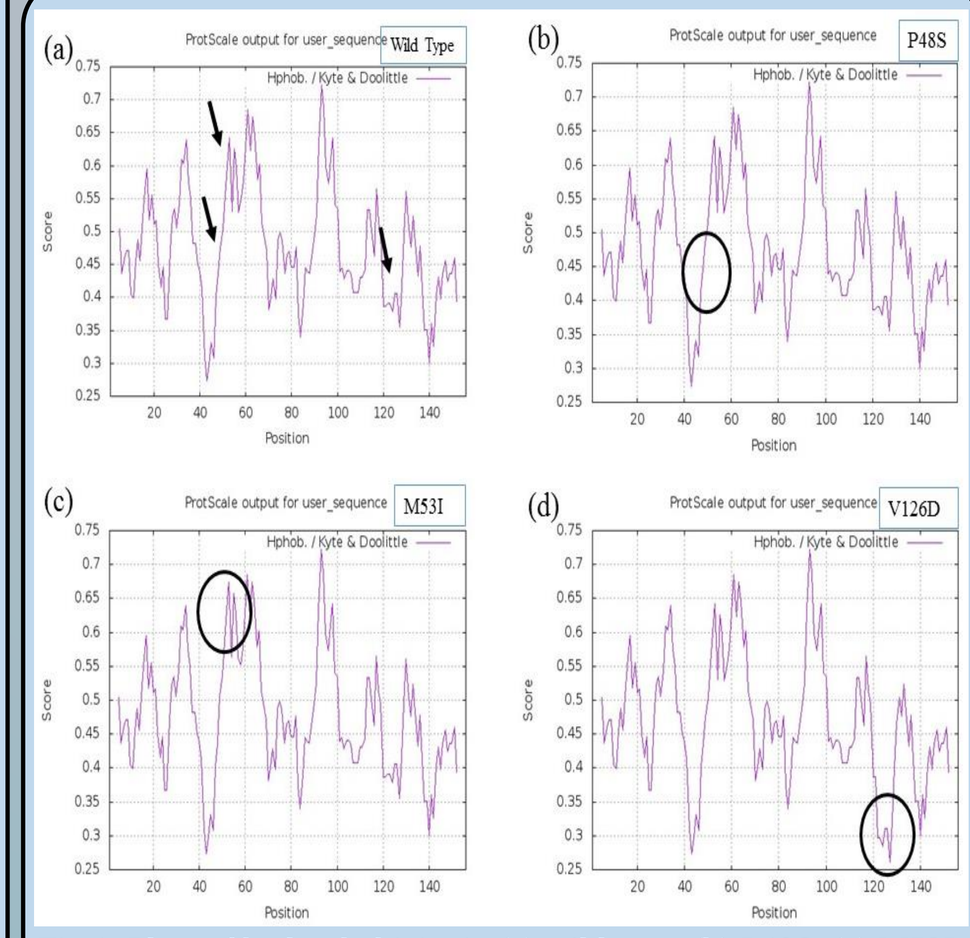

Fig 1: Plots of hydrophobicity in (a) wild type, (b) P48S, (c) M53I and (d) V126D.

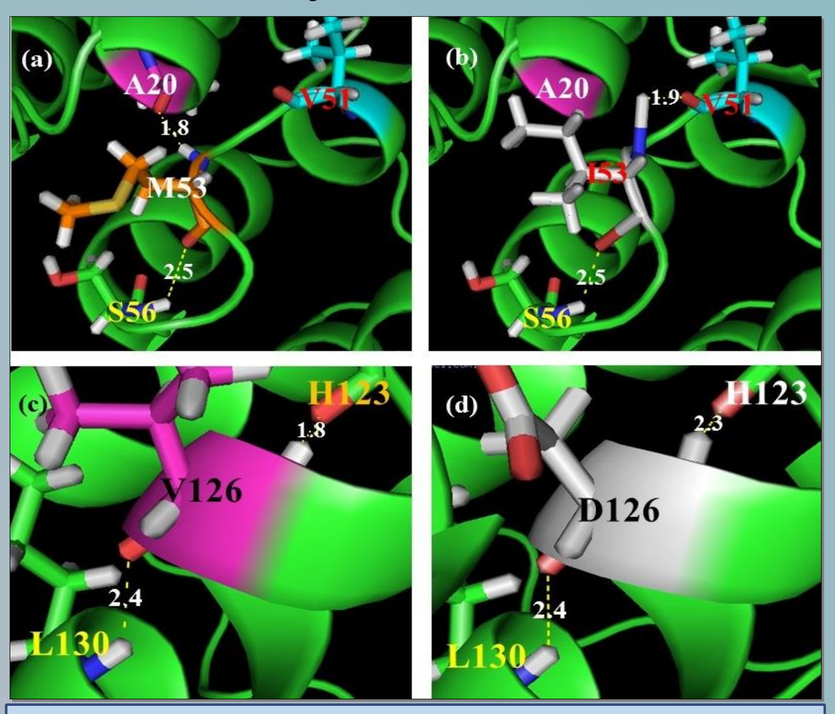

Fig 2: H-bond length in (a) wild type at position 53, (b) M53I substitution. H-bond length in (c) wild type at position 126, (d) V126D substitution.

As shown in TABLE 1, in the all of tools the V126D mutation has been identified as a damaging variant. At position 48, mutant residue is smaller than the wild type residue. The mutation will cause an empty space in the core of the protein. About M53I amino acid substitution, located on ANK 2, the wild type and mutant amino acids differ in size and the mutant residue is smaller than the wild type residue. About V126D amino acid substitution, located in ANK 4, there are differences in hydrophobicity (see TABLE 2), charge and size between the wild type (Valine/Neutral charge) and mutant amino acid (Aspartic acid/negative charge). So, the mutant residue can lead to protein folding problems. Also, the mutant residue is bigger than the wild type residue. The wild type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit. According to Fig. 1, we find that there are hydrophobicity alterations in M53I and V126D.

3D protein modeling:

As shown in Fig. 2, the polar contacts in M53I and V126D amino acid substitutions have been changed. But, there is no change in polar contact in P48S amino acid substitution. It should be mentioned that the association of V126D and M53I with melanoma [6,7] and the correlation of V126D with primary cervical lesions [8] have been reported in previous studies.

Conclusions

In this study, we investigated the pathogenicity of three nsSNVs in the *CDKN2A* gene (p16) using bioinformatics tools. According to this consideration, rs786204195 and rs104894095 may have damaging effects, but the harmfulness of rs104894098 is more certain. These mentioned facts have been found due to the existence of alterations in mutant protein structure, hydrophobicity and obtained prediction by bioinformatics tools.

References:

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