Evaluation of cancer related missense mutations in CENPH

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INTRODUCTION

CENPH is an essential component of the centromere and kinetochore complex and one of the constitutive kinetochore proteins [1]. During the cell cycle, CENPH localizes in the inner kinetochore plate with other constitutive kinetochore proteins [1]. CENPH is required for controlled kinetochore-microtubule plus-end dynamics and chromosome oscillations [2]. Loss of CENPH causes serious mitotic phenotypes, such as chromosome misalignment and multi-polar spindles [3].

Previous studies showed that CENPH has an important role in carcinogenesis of different cancers and is a promising prognostic marker. High CENPH expression has been shown in various cancers and CENPH has been proposed as a prognostic marker for patient survival of hepatocellular carcinoma, non-small cell lung cancer, renal cell carcinoma, nasopharyngeal carcinoma, breast cancer, and esophageal carcinoma [4-9]. In hepatocellular carcinoma cells knockdown of CENPH inhibited cell proliferation and induced apoptosis and growth arrest [10]. In renal cell carcinoma cell lines, CENPH knockdown caused a decrease in cell proliferation and an increase in apoptosis also inhibited cell growth, showing the importance of this protein in cancer progression [7].

Although detailed studies of CENPH expression alterations has been reported in human cancers, the effect of CENPH mutations in human cancers has not been studied in detail. In consideration of the importance of CENPH in carcinogenesis, it is necessary to investigate and classify CENPH mutations in...
human cancers. Therefore, in this study missense mutations that were identified in different cancers of CENPH were extracted and their potential effect was investigated.

MATERIALS and METHODS

Obtaining missense mutations from Catalogue of Somatic Mutations in Cancer (COSMIC) database
Missense mutations of CENPH that were observed in cancer samples were downloaded from the COSMIC v89 (https://cancer.sanger.ac.uk/cosmic, [11]).

Predicting the effect of missense mutations
PredictSNP1.0 (https://loschmidt.chemi.muni.cz/predictsnp1/, [12]) was used to predict the effect of missense mutations. This tool is a consensus classifier that integrates the predictions of in silico prediction tools. For prediction analysis, protein sequence of CENPH (NP_075060.1) in fasta format from NCBI database (https://www.ncbi.nlm.nih.gov, [13]) and missense mutations from COSMIC v89 [11], were submitted to PredictSNP1.0 [12]. As a result, the PredictSNP prediction and predictions of each integrated tool were reported for missense mutations as neutral or deleterious along with expected accuracies.

In silico prediction of CENPH protein structure
The complete protein structure of the CENPH protein was predicted using I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/, [14-16]). The model with highest C-score was selected for CENPH model. CENPH protein and predicted deleterious mutations were visualized by using UCSF Chimera (http://www.rbvi.ucsf.edu/chimera, [17]). Dunbrack rotamer library [18] was used to display rotamers for selected missense mutations in UCSF Chimera.

Structural effects of selected missense mutations on CENPH protein
The structural effects of p.R105W and p.V237F on CENPH protein were analyzed by using HOPE server (http://www.cmbi.ru.nl/hope/, [19]).

RESULTS

Mutations of CENPH from COSMIC database
Cancer associated CENPH mutations were investigated by using COSMIC database v89. The information for total 49 mutations; 34 missense, 7 coding silent, 1 nonsense substitutions and 3 frameshift deletions, 1 frameshift insertion and 3 unknown mutations, which had no information at the amino acid level were obtained for CENPH. The 34 missense mutations, which cause a change in amino acid of the protein, were selected for further analysis.

Prediction of deleterious mutations of CENPH
In order to assess the effect of 34 missense mutations on CENPH, PredictSNP1.0 tool was used. Of the 34 missense mutations, 18 mutations were predicted as deleterious, while 16 mutations were predicted as neutral by PredictSNP1.0 (Table 1).

| CDS Mutation | AA Mutation | Mutation ID (COSM) | PredictSNP prediction | Expected accuracy |
|--------------|-------------|--------------------|-----------------------|------------------|
| c.4G>A       | p.E2K       | 33151              | DELETERIOUS           | 0.51             |
| c.29C>T      | p.A10V      | 6393548            | NEUTRAL               | 0.83             |
| c.62G>T      | p.R21L      | 4638766            | NEUTRAL               | 0.74             |
| c.95A>G      | p.Q32R      | 6398012            | NEUTRAL               | 0.65             |
| c.116G>T     | p.R39L      | 6321194            | NEUTRAL               | 0.65             |
| c.140G>C     | p.R47T      | 7099599            | DELETERIOUS           | 0.55             |
| c.180G>A     | p.M60I      | 3856099            | NEUTRAL               | 0.83             |
| c.196G>A     | p.E66K      | 6864852            | DELETERIOUS           | 0.65             |
| c.235G>A     | p.E79K      | 1438372            | DELETERIOUS           | 0.65             |
| Mutation   | Amino Acid | ESPID   | Category       | Probability |
|------------|------------|---------|----------------|-------------|
| c.239C>T   | p.A80V     | 3856100 | NEUTRAL        | 0.83        |
| c.251A>T   | p.D84V     | 6171577 | NEUTRAL        | 0.83        |
| c.262G>C   | p.E88Q     | 6104363 | DELETERIOUS    | 0.65        |
| c.268G>A   | p.E90K     | 3072066 | DELETERIOUS    | 0.61        |
| c.313A>T   | p.R105W    | 6104362 | DELETERIOUS    | 0.87        |
| c.314G>A   | p.R105K    | 6900614 | DELETERIOUS    | 0.51        |
| c.314G>T   | p.R105M    | 1438373 | DELETERIOUS    | 0.72        |
| c.368C>T   | p.S123F    | 3920291 | NEUTRAL        | 0.63        |
| c.403G>C   | p.E135Q    | 7120230 | NEUTRAL        | 0.83        |
| c.414A>C   | p.K138N    | 328568  | NEUTRAL        | 0.63        |
| c.430C>A   | p.Q144K    | 6426334 | DELETERIOUS    | 0.55        |
| c.472A>G   | p.R158G    | 738326  | DELETERIOUS    | 0.65        |
| c.477G>T   | p.K159N    | 3429596 | DELETERIOUS    | 0.72        |
| c.553G>T   | p.D185Y    | 6398013 | DELETERIOUS    | 0.65        |
| c.568G>A   | p.E190K    | 7179206 | NEUTRAL        | 0.83        |
| c.570A>C   | p.E190D    | 1200752 | NEUTRAL        | 0.74        |
| c.581G>A   | p.R194K    | 4895150 | NEUTRAL        | 0.83        |
| c.587A>G   | p.K196R    | 3366136 | NEUTRAL        | 0.65        |
| c.588G>T   | p.K196N    | 1069685 | DELETERIOUS    | 0.61        |
| c.686A>G   | p.E229G    | 3366137 | DELETERIOUS    | 0.61        |
| c.691C>A   | p.P231T    | 7186878 | DELETERIOUS    | 0.61        |
| c.695C>A   | p.A232D    | 3669472 | NEUTRAL        | 0.74        |
| c.709G>T   | p.V237F    | 7314207 | DELETERIOUS    | 0.87        |
| c.730G>T   | p.V244F    | 3366138 | DELETERIOUS    | 0.52        |
| c.738G>T   | p.M246I    | 6171576 | NEUTRAL        | 0.74        |

**Visualization of the predicted deleterious mutations on CENPH protein structure**

In order to evaluate predicted deleterious missense mutations on CENPH protein, the protein structure of CENPH was predicted by using I-TASSER and visualized by using UCSF Chimera. The localizations of the residues, which carry predicted deleterious missense mutations, were shown on 3D CENPH protein model (Figure 1). According to distribution pattern the predicted missense mutations were scattered along the whole protein structure (Figure 1).
Figure 1. The localization of mutated residues, which carry predicted deleterious mutations, were shown in ball and stick representation on CENPH protein. The protein model of CENPH was generated by using I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/, [14-16]) and visualized by using UCSF Chimera (http://www.rbvi.ucsf.edu/chimera, [17]).

Visualization of p.R105W and p.V237F on CENPH protein

p.R105W and p.V237F were the two missense substitutions that were predicted as deleterious by PredictSNP with high expected accuracy; 0.87 (Table 1). p.R105W was the missense substitution of arginine at position 105 to tryptophan (Figure 2).

Figure 2. p.R105W mutation on CENPH protein. The original Arginine, R, was shown in ball and stick presentation and rotamers of mutated Tryptophan, W, was shown in wire presentation.
Cancer related missense mutations

p.V237F was the missense substitution of valine at position 237 to phenylalanine (Figure 3).

Figure 3. p.V237F mutation on CENPH protein. The original Valine, V, was shown in ball and stick presentation and rotamers of mutated Phenylalanine, F, was shown in wire presentation.

Structural effects of p.R105W and p.V237F on CENPH protein

In order to detect the structural effects of missense substitutions of p.R105W and p.V237F, HOPE server [19] was used. According to HOPE results, when the amino acid properties of wild type and mutant amino acid was considered, for the p.R105W substitution, the charge of the wild type residue was lost after the substitution. The mutant residue was bigger than the wild type residue. The wild type and mutant residue also differed in hydrophobicity. For the p.V237F substitution, the wild type and mutant amino acids differed in size.

DISCUSSION

In this study, the missense mutations of CENPH that were observed in different cancers were extracted and the effects of these mutations were investigated. The kinetochore is a proteinaceous assembly that connects centromeric DNA and spindle microtubules and is vital for chromosome segregation [20]. During mitosis, chromosome segregation problems stimulates aneuploidy, chromosome instability and tumorigenesis [21]. CENPH is one of the constitutive kinetochore proteins and is necessary for kinetochore-microtubule plus-end dynamics and chromosome oscillations [1, 2]. Knockdown of CENPH produces chromosome misalignment and multi-polar spindles [3]. Increased CENPH expression has been shown in different cancers and proposed as a promising prognostic marker [4-9]. Although there were studies in the literature about the aberrant expression of CENPH in cancers, studies searching the effect of CENPH mutations associated with cancers are needed. According to results of this study, 18 missense mutations were predicted as deleterious for CENPH (Table 1). In order to evaluate the localization of deleterious missense mutations on 3D protein, human CENPH protein was modeled and locations of predicted missense mutations were visualized on CENPH. On the 3D protein structure, the predicted deleterious missense mutations were displayed a scattered pattern instead of accumulating in one region (Figure 1). Interestingly R105 position carried three different substitutions, p.R105W, p.R105K and p.R105M that were all predicted as deleterious (Table 1).

In this study, two missense mutations, p.R105W and p.V237F, were predicted as deleterious with high
In this study, 3D CENPH protein was generated by an in silico method. Although, currently in silico protein modeling techniques predict the protein structures with high accuracy, they have also limitations and drawbacks [23]. Therefore, it should be noted that the created protein model of CENPH might not fully reflect the original 3D protein.

In addition, since this study was performed with computational methods, additional experimental methods are needed to validate the pathogenicity and phenotypic associations of predicted deleterious mutations.

CONFLICT OF INTEREST

There is no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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