RESEARCH ARTICLE

Binding Pattern Elucidation of NNK and NNAL Cigarette Smoke Carcinogens with NER Pathway Enzymes: an Onco-informatics Study

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Abstract

Cigarette smoke derivatives like NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol) are well-known carcinogens. We analyzed the interaction of enzymes involved in the NER (nucleotide excision repair) pathway with ligands (NNK and NNAL). Binding was characterized for the enzymes sharing equivalent or better interaction as compared to +Ve control. The highest obtained docking energy between NNK and enzymes RAD23A, CCNH, CDK7, and CETN2 were -7.13 kcal/mol, -7.27 kcal/mol, -8.05 kcal/mol and -7.58 kcal/mol respectively. Similarly, the highest obtained docking energy between NNAL and enzymes RAD23A, CCNH, CDK7, and CETN2 were -7.46 kcal/mol, -7.94 kcal/mol, -7.83 kcal/mol and -7.67 kcal/mol respectively. In order to find out the effect of NNK and NNAL on enzymes involved in the NER pathway applying protein-protein interaction and protein-complex (i.e. enzymes docked with NNK/NNAL) interaction analysis. It was found that carcinogens are well capable to reduce the normal functioning of genes like RAD23A (HR23A), CCNH, CDK7 and CETN2. In silico analysis indicated loss of functions of these genes and their corresponding enzymes, which possibly might be a cause for alteration of DNA repair pathways leading to damage buildup and finally contributing to cancer formation.

Keywords: NER pathway enzymes - NNK - NNAL - cigarette smoke carcinogens - docking

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Introduction

Lung cancers are powerfully linked with cigarette smoke carcinogens like NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol) (Xue et al., 2014). One of the preliminary critical actions is most likely damage of the hereditary material (DNA) by a cigarette smoke carcinogen. This damage can, beneath the definite status, be repaired by cellular DNA repair mechanisms (Raphael Ceccaldi et al., 2015). Though, if not repaired, cells will try to duplicate their DNA during cell division, but are obstructed by the damage and will do fault duplication progression leading to gene mutations brought onto a trail of uncontrolled cell division leading to a tumor growth. Studies show that in ordinary cells, NER removes numerous types of DNA lesions, defending cell integrity (Rouillon et al., 2011).

However, in cancer cells uncovered to DNA damaging compounds that alter the DNA helix or form unwieldy injuries to the genome, NER take part in the managing the damage, consequently protecting cancer cells from fatality (Nouspikel, 2009). But NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol) can alter the biological activity of NER repair enzymes. Therefore, in order to execute our hypothesis, we have selected 17 enzymes involved in NER pathways and their interaction with cigarette smoke carcinogens NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol) can alter the biological activity of NER repair enzymes. Therefore, in order to execute our hypothesis, we have selected 17 enzymes involved in NER pathways and their interaction with cigarette smoke carcinogens NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol) can alter the biological activity of NER repair enzymes.
RAD23 homolog A) involved in nucleotide excision repair and is thought to be functionally equivalent for RAD23B in global genome nucleotide excision repair (GG-NER) by association with XPC. Two human homologs of Rad23 are functionally interchangeable in complex formation and stimulation of XPC repair activity (Sugasawa et al., 1997).

CCNH (cyclin H) regulates CDK7, the catalytic subunit of the CDK-activating kinase (CAK) enzymatic complex. CAK activates the cyclin-associated kinases CDK1, CDK2, CDK4 and CDK6 by threonine phosphorylation. CDK7 (Cyclin-dependent kinase 7) required for DNA-bound peptides-mediated transcription and cellular growth inhibition DNA-Bound peptides control the mRNA transcription through CDK7 (Lu X et al., 2009) and CETN2 (Centrin-2) Involved in global genome nucleotide excision repair (GG-NER) by acting as a component of the XPC complex. Cooperatively with RAD23B appears to stabilize XPC. Centrosome protein centrin2/caltractin1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair (Araki M et al., 2001; S.Matsumoto et al., 2015).

Computational tools such as molecular docking are important to understand the binding capabilities of NNK and NNAL with enzymes involved in NER pathways (Xia et al., 2012). It has never been explored through in silico approaches. Therefore, we used protein-protein docking to know the functional loss of the enzymes due to their interaction with NNK and NNAL. In order to perform protein-protein interaction it is necessary to find out the co-operated functional enzymes encoded by genes using STRING 9.0.5 database (Szklarczyk et al., 2011) and their 3D structures. At last the comparative analysis (Protein-Complex*) was completed by ZDOCK protocol using Discovery Studio Client 2.5 (Accelrys Software Inc, 2013).

Materials and Methods

Preparation of ligand structures

Ligand file of NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol) were downloaded in .mol format (Figure: 1 and Figure: 2) from ChemSpider Chemical Database (Harry et al., 2010). These files could not directly use by Autodock 4.2 tools (Morris et al., 2009) thus; we have to convert it into .pdb files and also further the ligands were submitted for CHARMm (Brooks et al., 1983; 2009) energy minimization protocol in Discovery Studio Client 2.5.

Preparation of protein structures

The structures of enzymes involved in the NER pathways were obtained from Protein Data Bank (Berman et al., 2000) (Table 1). Published structures were edited to remove HETATM and water molecule using Discovery Studio Client 2.5. Energy minimization was performed by the implementation of CHARMm force field (D.T Mirijanian et al., 2014) after addition of hydrogen atoms to the selected enzymes using Accelrys Discovery studio client 2.5.

Docking studies

Molecular Docking studies were performed to analyze the binding affinity of NNK/NNAL with enzymes involved NER pathways. Autodock (Version 4.2) suite (Morris et al., 1998; 2009) and Cygwin interface was used in the Microsoft Windows 7 professional, operating System on Intel® Xeon® Processor E3-1220 v3 (Quad Core, 3.10GHz Turbo, 8MB) and 256GB 2.5inch Serial ATA Solid State Drive Dell Precision T7700 Workstation was used to dock the NNK/NNAL on binding site of the enzymes. Molecular docking methods followed by the searching the best conformation of enzymes and carcinogens complex on the basis of binding energy. Water molecules were removed from the 3D X-ray crystallography structures of enzymes before docking and hydrogen atoms were added to all target enzymes. Kollman united charges and salvation parameters were added to the enzymes. Gasteiger charge was added to the ligands. Grid box was set to cover the maximum part of enzymes and ligands. The values were set to 60×60×60 Å in X, Y and Z axis of a grid point. The default grid points spacing was 0.375 Å. Lamarckian Genetic Algorithm (LGA) (Goodsell et al., 1996; Tsai et al., 2012) was used for enzymes-ligands flexible docking calculations. The LGA parameters like population size (ga_pop_size), energy evaluations (ga_num_generation), mutation rate, crossover rate and step size were set to 150, 2500000, 27000, 0.02, 0.8 and 0.2 Å, respectively. The LGA runs were set at 50 runs. All obtained conformations of enzymes and ligand complex were analyzed the interactions and binding energy of the docked structure using Discovery Studio 2.5 molecular visualization software.

Protein-protein interaction analysis

We found the interacting proteins (used as ligands) of selected enzymes using STRING 9.0.5 database that predict interacting interactions incorporate direct (physical) and indirect (functional) associations derived

![Figure 1. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone, PubChem Compound ID- 47289, ChemSpider ID 43038](image1)

![Figure 2. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol, PubChem Compound ID- 104856, ChemSpider ID- 94646dd](image2)
from four sources, i.e. genomic context, high throughput experiments (conserved) co-expression and previous knowledge of proteins against your query (Figure:5 A,B,C and D). We used discovery studio Client 2.5 for Zdock (Dock Proteins) Protocol. Zdock scores obtained for both Protein-Protein interactions as well as for Protein-Complex (ligand protein+NNK/NNAL) interaction.

Zdock calculations

Discovery studio Client 2.5 was used to complete protein-protein docking using ZDOCK is an initial stage, rigid body molecular docking algorithm that uses a fast Fourier transform (FFT) algorithm to improve performance for searching in translational space (Chen et al., 2003; Pierce et al., 2014). All of the available structures from PDB were used to calculate the docking poses and the structures obtained were subjected to energy minimization using the smart minimize algorithm (Max steps 200, RMS gradient 0.01) in the program Discovery studio 2.5. The resulting Zdock scores with the highest value were used as appropriate conformational pose (Jamal et al., 2012).

Results and Discussion

In the achievement of the current investigation molecular docking techniques were adopted to explore the binding capabilities of NNK and NNAL with enzymes encoded by respective genes of NER pathways. Primarily the 1IRD (Crystal Structure of Human Carbonmonoxy-Haemoglobin at 1.25 Å Resolution) was used as a positive control and 3C19 (Human heat shock factor-binding protein 1) as a negative control to validate our docking analysis. Molecular interaction results of these enzymes showed that 1IRD docked with NNK, observed binding energy was -6.68 Kcal/Mol, it docked with NNAL and observed binding energy was -6.31 Kcal/Mol. 3C19 docked with NNK with the experimental binding energy was -6.31 Kcal/Mol. 3CI9 energy was -6.68 Kcal/Mol, it docked with NNAL and showed that 1IRD docked with NNK, observed binding analysis. Molecular interaction results of these enzymes protein 1) as a negative control to validate our docking control and 3CI9 (Human heat shock factor-binding protein) was used as a positive control for searching in translational space (Chen et al., 2003; Pierce et al., 2014). All of the available structures from PDB were used to calculate the docking poses and the structures obtained were subjected to energy minimization using the smart minimize algorithm (Max steps 200, RMS gradient 0.01) in the program Discovery studio 2.5. The resulting Zdock scores with the highest value were used as appropriate conformational pose (Jamal et al., 2012).

Table 1. NNK and NNAL Docked with Enzymes Involved in NER Pathway

| S. No. | Gene’s Name | PDB ID | Accession Code | GenBank | Uniprot | Docking Energy (vdW + Hbond + desolv Energy ) |
|--------|-------------|--------|----------------|---------|---------|---------------------------------------------|
|        |             |        |                |         |         | Docked with NNK | Docked with NNAL |
| 1.     | RPA2        | 1DPU   | NM_002946      | NP_002937 | P15927 | -6.53 kcal/mol | -6.19 kcal/mol |
| 2.     | RAD23A (HR23A) | 1DVO   | NM_005053      | NP_005044 | P54725 | -7.13 kcal/mol | -7.46 kcal/mol |
| 3.     | MT1         | 1G25   | NM_002431      | NP_002422 | P51948 | -6.06 kcal/mol | -5.98 kcal/mol |
| 4.     | CCNH        | 1KXU   | NM_001239      | NP_001230 | P51946 | -7.27 kcal/mol | -7.94 kcal/mol |
| 5.     | RAD23B (HR23B) | 1P1A   | NM_002874      | NP_002865 | P54727 | -6.46 kcal/mol | -7.01 kcal/mol |
| 6.     | CDK7        | 1U2A   | NM_001799      | NP_001790 | P50613 | -8.05 kcal/mol | -7.83 kcal/mol |
| 7.     | LIG1        | 1X9N   | NM_000234      | NP_000225 | P18858 | -6.88 kcal/mol | -7.46 kcal/mol |
| 8.     | XPA         | 1XPA   | NM_000380      | NP_000371 | P23025 | -6.99 kcal/mol | -6.82 kcal/mol |
| 9.     | GTF2H2      | 1Z0O   | NM_001515      | NP_001506 | Q13888 | -6.74 kcal/mol | -7.43 kcal/mol |
| 10.    | CETN2       | 1ZMQ   | NM_004344      | NP_004335 | P41208 | -7.58 kcal/mol | -7.67 kcal/mol |
| 11.    | ERCC1       | 2AII   | NM_001983      | NP_973730 | Q7Z7F5 | -6.74 kcal/mol | -6.54 kcal/mol |
| 12.    | ERCC4 (XPF) | 2AQ0   | NM_005236      | NP_005227 | Q92889 | -6.41 kcal/mol | -6.23 kcal/mol |
| 13.    | GTF2H5 (TTDA) | 2NJN  | NM_207118      | NP_997001 | Q6ZYL4 | -6.27 kcal/mol | -5.33 kcal/mol |
| 14.    | XPC         | 2O8H   | NM_003800      | NP_003871 | P23025 | -6.25 kcal/mol | -6.08 kcal/mol |
| 15.    | GTF2H1      | 2KRN   | NM_005316      | NP_005307 | P32780 | -6.46 kcal/mol | -5.83 kcal/mol |
| 16.    | ERCC3 (XPB) | 4ERN   | NM_000122      | NP_000113 | P19447 | -4.28 kcal/mol | -5.56 kcal/mol |
### Table 2. Docking Studies of NNK and NER Pathways Enzymes Interaction

| S.No. | Enzymes    | PDB ID | H-Bonds | H-Bonds Distance (Å) | Residues involved in Hydrophobic region | Docking Energy (vdW + Hbond + desolv Energy) kcal/mol | Inhibition Constant (uM) |
|-------|------------|--------|---------|----------------------|-----------------------------------------|------------------------------------------------------|-------------------------|
| 1.    | RAD23A (HR23A) | 1DV0   | A:GLN21:HE21 - :UNK0:N4 | 2.40437 | Phe14,Ser17,Leu18,Gln21,Ala22, Phe36,Leu37,Leu38,Gln40,Asn41, Phe42,Asp43 | -7.13 | 112.03 |
|       |            |        | A:GLN21:HE21 - :UNK0:O2 | 1.8656 |                            |                                                      |                         |
| 2.    | CCNH       | 1KXU   | A:ASP202:HN - :UNK0:N4 | 2.32473 | 31                         | -7.27 | 91.24 |
|       |            |        |                        |         | Arg23,Met54,Cys57,Lys58,Glu61 ,Phe87,Lys88,Tyr91,Leu200, Thr 201,Asp202,Leu205,Leu258,Lys2 61,Tyr262 |                                                      |                         |
|       |            |        | A:ASP202:HN - :UNK0:O2 | 1.94374 |                            |                                                      |                         |
| 3.    | CDK7       | 1UA2   | A:LYS139:HZ3 - :UNK0:O1 | 1.67265 | Gly21,Gln22,Phe23,Lys41,His135 ,Asp137,Lys139,Asn142,Ala154,A sp155,Phe156,Gly157,Lys160,Ser 161,Phe162,Thr175 | -8.05 | 26.15 |
|       |            |        | A:ASN142:HD22 - :UNK0:O1 | 2.26816 |                            |                                                      |                         |
|       |            |        | A:ASN142:HD22 - :UNK0:O1 | 2.05534 |                            |                                                      |                         |
|       |            |        | A:SER161:HG - :UNK0:N4 | 1.8268  |                            |                                                      |                         |
|       |            |        | A:SER161:HG - :UNK0:O2 | 2.24771 |                            |                                                      |                         |
| 4.    | CETN2      | 1ZMZ   | A:ARG18:HN - :UNK0:N4 | 2.231   | Gln15,Arg16,Lys17,Arg18,Met19, Leu25,Gln29,Lys30,Gln31,Ile33,Arg34,Phe86,Leu90 | -7.58 | 49.61 |
|       |            |        | A:ARG18:HN - :UNK0:O2 | 1.7428  |                            |                                                      |                         |
|       |            |        | A:MET19:HN - :UNK0:O2 | 1.87497 |                            |                                                      |                         |
|       |            |        | A:LYS30:H2Z - :UNK0:O1 | 2.09563 |                            |                                                      |                         |

Figure 3. (A) 1DV0 (RAD23A (HR23A)) Docked with NNK (in Purple color) (B) 1KXU (CCNH) Docked with NNK (C) 1UA2 (CDK7) Docked with NNK (D) 1ZMZ (CETN2) Docked with NNK (in Purple color) and the Hydrogen Bonds Shown by Green Dotted Lines. All graphics generated by discovery studio visualizer.

Figure 4. (A) 1DV0 (RAD23A (HR23A)) Docked with NNAL (in Purple color) (B) 1KXU (CCNH) Docked with NNK (C) 1UA2 (CDK7) Docked with NNAL (D) 1ZMZ (CETN2) Docked with NNAL (in Purple color) and Hydrogen Bonds Shown by Green Dotted Lines. All graphics generated by discovery studio visualizer.
Gly157, Lys160, Ser161, Phe162, and Thr175 amino acids residues. The estimated inhibition constant of NNK and CDK7 docked complex was 26.15 μM (Table 2 Figure: 3 C). The CETN2 involved in the building of 4 hydrogen bonds A: ARG18: HN - :UNK0:N4; A: ARG18: HN - :UNK0:O2; A: MET19: HN - :UNK0:O2, and A: LYS30:HZ2 - :UNK0:O1 with the distance of 2.231 Å, 1.7428 Å, 1.87497 Å and 2.09563 Å respectively. The hydrophobic pocket characterized by the occurrence of Gly15, Arg16, Lys17, Arg18, Met19, Leu25, Gln29, Lys30, Gln31, Ile33, Arg34, Phe86, and Leu90 amino acids residues. The estimated inhibition constant of NNK and CETN2 docked complex was 46.61 μM (Table 2 Figure: 3 D).

Furthermore, the active site characterization analysis of top four enzymes also revealed that NNAL and NER enzymes shown that RAD23A involved in the building of 3 hydrogen bonds A: GLN21:HE21 - :UNK0:O2, A: ASN41:HD21 - :UNK0:N4, and: UNK0:H24 - A: PHE42: O with the distance of 2.26011 Å, 2.18982 Å, and 2.03172 Å respectively. The hydrophobic pocket characterized by the occurrence of Arg14, Leu18, Gln21, Ala22, Phe36, Leu37, Gln40, Asn41, Phe42, and Asp43 amino acid residues. The estimated inhibition constant of NNAL and RAD23A docked complex was 67.58 μM (Table 3 Figure: 4 A). The CCNH involved in the building of 4 hydrogen bonds A: LYS88:HZ2 - :UNK0:O1, A: ASP202: HN - :UNK0:N5, A: ASP202: HN - :UNK0:O2, and: UNK0:H24 - A: GLU61:OE1 with the distance of 2.26011 Å, 2.14097 Å, 2.16487 Å and 1.85083 Å, respectively. The hydrophobic pocket characterized by the occurrence of Arg23, Met54, Cys57, Lys58, Glu61, Lys88, Arg23, Met54, Cys57, Leu81, and Asp202 amino acids residues. The estimated inhibition constant of NNAL and CCNH docked complex was 29.57 μM (Table 3 Figure: 4 B). The CETN2 docked complex was 43.67 μM (Table 3 Figure: 4 C).

### Table 3. Docking Studies of NNAL and NER Pathways Enzymes Interaction

| S.No. | Enzymes | PDB ID | H-Bonds | H-Bonds Distance (Å) | Residues involved in Hydrophobic region | Docking Energy (vdW + Hbond + desolv Energy) kcal/mol | Inhibition Constant (μM) |
|-------|---------|--------|---------|---------------------|----------------------------------------|------------------------------------------------------|-------------------------|
| 1.    | RAD23A  | 1DV0   | A:GLN21:HE21 - :UNK0:O2 | 2.07489 | Phe14,Leu18,Gln21,Ala22,Phe36, -7.46 kcal/mol | 67.58 μM |
|       |         |        | A:ASN41:HD21 - :UNK0:N4 | 2.18982 | Leu37,Gln40,Asn41,Phe42,Asp43 |
|       |         |        | :UNK0:H24 - A: PHE42: O | 2.03172 |
| 2.    | CCNH    | 1KXU   | A:LYS88:HZ2 - :UNK0:O1 | 2.26011 | Arg23,Met54,Cys57,Lys58,Glu61, -7.94 kcal/mol | 29.57 μM |
|       |         |        | A:ASP202:HN - :UNK0:N5 | 2.14097 | Phe87,Lys88,Tyr91,Leu92,Leu200, |
|       |         |        | A:ASP202:HN - :UNK0:O2 | 2.16487 | Thr201,Asp202 |
|       |         |        | :UNK0:H24 - A:GLU61:OE1 | 1.85083 | Leu205,Leu258,Tyr262 |
| 3.    | CDK7    | 1UA2   | A:LEU138:HN - :UNK0:N5 | 1.96313 | His135,Arg136,Asp137,Leu138,Lys139, -7.83 kcal/mol | 39.55 μM |
|       |         |        | A:LEU138:HN - :UNK0:O2 | 2.04471 | Phe162,Thr175,Arg176,Tyr178,Arg179 |
|       |         |        | :UNK0:H24 - A:ASP137:OD1 | 1.84497 | Leu183,Val194,Ala198 |
|       |         |        | :UNK0:N5 - A:LEU138:O | 3.00052 |
|       |         |        | :UNK0:N5 - A:Tyr178:O | 3.13345 |
| 4.    | CETN2   | 1ZMZ   | A:ARG18:HN - :UNK0:N5 | 2.0811 | Arg16,Lys17,Arg18,Met19,Leu25, -7.67 kcal/mol | 43.67 μM |
|       |         |        | A:ARG18:HN - :UNK0:O2 | 2.14078 | Gln29,Lys30,Ile33,Arg34,Phe86, |
|       |         |        | A:MET19:HN - :UNK0:N5 | 1.87932 | Leu90 |
|       |         |        | A:MET19:HN - :UNK0:O2 | 2.17551 |
|       |         |        | A:LYS30:HZ2 - :UNK0:O1 | 1.84969 |
|       |         |        | :UNK0:H24 - A:MET19:O | 2.41349 |
Table 4. NNK and NNAL Binding to the Enzymes Reduces their Normal Functions after Analyzing the ZDOCK Scores

| S. No. | Selected Genes | PDB ID | Interacted enzymes | PP interaction of NNK with enzymes | ZDOCK Score | PP interaction of NNAL with enzymes | ZDOCK Score |
|--------|----------------|--------|--------------------|------------------------------------|-------------|-------------------------------------|-------------|
| 1.     | RAD23A (HR23A) | 1DV0   | 2KDE               | 1DV0 vs 2KDE                       | 14.58       | 1DV0 vs 2KDE                        | 14.58       |
|        |                |        |                    |                                    |             |                                    |             |
|        |                |        |                    |                                    |             |                                    |             |
| 2.     | CCNH           | 1KXU   | 1UA2               | 1KXU vs 1UA2                       | 14.24       | 1KXU vs 1UA2                        | 14.24       |
|        |                |        |                    |                                    |             |                                    |             |
| 3.     | CDK7           | 1UA2   | 1KXU               | 1UA2 vs 1KXU                       | 14.96       | 1UA2 vs 1KXU                        | 14.96       |
|        |                |        |                    |                                    |             |                                    |             |
| 4.     | CETN2          | 2GGM   | 1ZMZ               | 1ZMZ vs 2GMM                       | 15.92       | 1ZMZ vs 2GMM                        | 15.92       |

Figure 5. (A) CCNH Interacted with CDK7 (B) CDK7 Interacted with CCCNH (C) CETN2 Interacted with XPC (D) RAD23A Interacted with PSMD4. All interaction networks of selected enzymes obtained from STRING database

Table 3 Figure: 4 D). CETN2 involved Val194, and Ala198 amino acid residues. The estimated inhibition constant of NNAL and CETN2 complex was 43.67 uM (Table 3 Figure: 4 D).

In the further analysis, the protein-protein docking was adopted using ZDOCK protocol in Discovery Studio Client 2.5. Initially, we have found out the cooperated the enzymes encoded by genes for four selected enzymes, i.e. RAD23A, CCNH, CDK7, and CETN2 by STRING 9.0.5 database. The found Best closely related enzymes for RAD23A PDB ID: 1DV0 (RAD23 Homolog A) was PDB ID: 2KDE (PSMD4 MCB1, 26S proteasome non-ATPase regulatory subunit 4), for CCNH PDB ID: 1KXU (CDK-Activating Kinase Complex Subunit) was PDB ID: 1UA2 (CDK7, Cell division protein kinase 7), for CDK7 PDB ID: 1UA2 (Cyclin H) was PDB ID: 1KXU (CDK-Activating Kinase Complex Subunit) and for CETN2 PDB ID: 1ZMZ (Centrin, EF-Hand Protein, 2) was PDB ID: 2GGM (XPC, DNA-repair protein complementing XP-C cells) (Figure 5 A, B, C and D). Later on we run ZDOCK program for Protein-Protein Docking with Protein-Complex docking analysis.

The obtained Zdock scores 1DV0 vs 2KDE was 14.58, 1DV0+NNK vs 2KDE was 13.74, 1KXU vs 1UA2 was 14.24, 1KXU+NNK vs 1UA2 was 13.08, 1UA2 vs 1KXU was 14.96, 1UA2+NNK vs 1KXU was 13.84, 1UA2+NNAL vs 1KXU was 13.68, 1ZMZ vs 2GMM was 15.92 and 1ZMZ+NNK vs 2GMM was 15.40 (Table 4). Similarly, obtained Zdock scores 1DV0 vs 2KDV was 14.58, 1DV0+NNAL vs 2KDE was 13.76, 1KXU vs 1UA2 was 13.84, 1UA2 vs 1KXU was 14.58, 1UA2+NNAL vs 1KXU was 13.84, 1UA2+NNAL vs 1KXU was 13.68, 1ZMZ vs 2GMM was 15.92 and 1ZMZ+NNAL vs 2GMM was 15.55 (Table 4). The results shown that Zdock score of protein complex (contain enzymes and cigarette smoke carcinogens conformation) interaction were higher than protein-protein interaction. Analysis clearly revealed that when NNK/NNAL interacts with NER enzymes their metabolic activity to form complex with its cooperated enzymes reduces significantly. Thus, NNK and NNAL were capable to damage the DNA repair machinery and its will lead to the functional loss of NER enzymes encoded by genes RAD23A, CCNH, CDK7, and CETN2.
Conclusion

This hypothesis able to provide better understanding to explore the molecular interaction of NNK and NNAL with enzymes involved in NER pathways. It is also helpful to understand the biological insights of NNK and NNAL binding efficacy in the progression of cancer. The study revealed that the enzymatic activity of these enzymes RAD23A, CCNH, CDK7, and CETN2 affected by NNK and NNAL. Therefore, the possibility of DNA damage will be increased because these enzymes have an important role in the DNA damage control. Once the DNA repair machinery altered due to interaction of cigarette smoke carcinogens NNK and NNAL, the whole biological process will lead to uncontrolled tumor growth and finally cancer will be developed. For the further confirmation of study the in vivo and in vitro validation needed.

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