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In silico Analysis suggests Anti-cancer Drugs Interactions and their Downstream effects with Protein targets are similar in Humans and Animal models

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Abstract: Increased production of new anticancer drugs has developed demand for human animal models for test subjects. In the present piece of work, a computational approach was used to study the drug- protein interaction in animal models and humans of Geneticin G418, Plitidepsin, Desmethyl pateamine A. The animal models and human proteins were analysed for the similarity in protein structure by multiple sequence alignment and structural analysis. Previous studies had reported interaction of specific amino acid residues in the protein target with the drug. FoldX was performed to mutate the drug binding residues, that were different in animal models compared to humans to know the drug-protein interaction in specific animal models, We docked the drug using autodock to the target proteins, and studied the binding properties of drugs: Geneticin G418 bound to Ornithine Decarboxylase, Plitidepsin bound to eEF1A-II, and desmethyl pateamine A bound to eIF4A-I. Structural analysis of the drug with the animal model protein and its interaction were visualised in pyMOL. The target protein functional pathway was also checked for their conservation in both humans and human animal models. The protein Ornithine Decarboxylase of Monkey showed 98.05 percent similarity, eEF1A-II and eIF4A-I of Monkey Rabbit showed 100 percent similarity to human proteins respectively making them effective animal models. This computational analysis allows us to analyse which animal model will be best for testing specific drugs that will help choose appropriate animal test subjects.

Keywords: Geneticin G418, Plitidepsin, Desmethyl pateamine A, eIF4A-I, eEF1A-II, Ornithine Decarboxylase, Human animal models, Cancer.

1. INTRODUCTION

Cancer is caused by abnormal and uncontrollable cell growth. These abnormal cells develop into tumors and become fatal to the body. Cancer can be caused by different carcinogens like hazardous chemicals, radiation, and stochastic mutations. Research on cancer requires deep knowledge and understanding of disease to develop any new novel drugs. There are various types of drugs which are developed for cancer treatment, these drugs have specific target proteins on which they act (9). In silico computational studies offer cellular protein and genetic information. This can be used for non-clinical studies which help us know the efficiency of specific drugs in animal models. Drugs that were already in use like Dinaciclib with target protein CDK2/5/9 Romidepsin (1) with Histone deacetylase, Niraparib (2) with PARP1/2 were tested with the hypothesis of in silico analysis showing mice as effective and suitable animal model.

In the present work we choose the following drugs: Desmethyl pateamine A (DMPatA), Plitidepsin, and Geneticin (G418) which are in preclinical trials were selected which are anticancer drugs that target the translational and transcription proteins eIF4A-I, eEF1A-II, and Ornithine Decarboxylase respectively. eIF4A-I is an ATP-dependent RNA helicase involved in the translation process by inhibiting the malignancies (3). eEF1A-II is involved in the translation process responsible for delivering aminoacylated tRNAs to the A site of the ribosome (4). And Ornithine Decarboxylase is helpful in catalysing the biosynthesis of the proteins (5).Testing any potential drug candidates on animal models is a compulsory requirement to know the safety and effectiveness. Monkey, rabbit, mouse, rat, frog, and Zebrafish animal models are widely used in cancer research due to their low cost, availability, and diversity of immunocompetent and immunodeficient strains(8)(9)(13). For analysis, MSA was performed to know the similarity of the target proteins of the above mentioned drugs in animal models and humans. We found that most of the amino acids were identical or replaced with similar amino acids showing they were structurally not exactly similar but drug binding sites were highly conserved.

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Structures of the target proteins were analsysed in UCSF Chimera. We mutated for the known drug binding residues using FoldX to study the whether the drug interactions with the target protein are similar in human and animal models. Autodock was performed in UCSF Chimera with Autodock vina option to study drug-protein bonding interactions. From the analysis, we found that for the drug DMPatA; rabbit, mouse and rat, Plitidepsin; rabbit, and monkey, Geneticin most suitable animal model was Monkey. And to study the similarity in the downstream effects of the drug targeted protein we checked for conservation for downstream effector proteins. DMPatA inhibits translation by blocking the RNA. In Plitidepsin VEGF and FAS pathways that leads to apoptosis (10) were checked for conservation to show they are similar in humans and animal models. And again the most conserved sequences were found in monkey animal model.

2. MATERIALS AND METHODS

2.1 Retrieval of Sequences

The proteins eIF4A1, eEF1A2 and Ornithine Decarboxylase sequences of humans were retrieved from sequence databases Uniprot (www.uniprot.org), and NCBI (www.ncbi.nlm.nih.gov) and by using the retrieved human sequence BLASTp (Blosum62 substitution matrix) (blast.ncbi.nlm.nih.gov/Blast.cgi) was performed to find other animal models sequences.

2.2 Structural comparison by Sequence alignment

Sequential comparisons of proteins eIF4A1, eEF1A2 and Ornithine Decarboxylase between human and other animal models were performed by aligning the sequences in Clustal Omega multiple sequence alignment tool (www.ebi.ac.uk/Tools/msa/clustalo/), to know the sequence similarity between human and animal models. Based on the previous studies, the drug binding amino acid residues were identified and checked for their conservation.

2.3 Structure templates searching and structural mutations

The sequences of the human eIF4A1 (P60842) and eEF1A2 (Q05639) were used as query in BLASTp from the Uniprot site (Blosum 62 substitution matrix) to retrieve sequences from different animal models. From the results of BLASTp, only reviewed results were filtered out, which had PDB or alpha fold predicted structures. The structures were viewed in Protein Data Bank to select the similar structures based on their chains and resolutions in monkey, rabbit, mouse, rat, frog, zebrafish animal models and downloaded in the PDB format.

Table 1. Structures retrieved through BLASTp

PDB ID	STRUCTURAL RESOLUTION	CHAINS IN STRUCTURE	SOURCES
Human (*Homo sapiens*) eIF4A1 protein structures			
2G9N	2.25 Å	A/B	PDB
2ZU6	2.80 Å	A/C/D/F	PDB
3EIQ	3.50 Å	A/D	PDB
5ZBZ	1.31 Å	A	PDB
5ZC9	2.00 Å	A	PDB
2G9N	2.25 Å	A/B	PDB
Mouse (*Mus musculus*) eIF4A1 protein structure			
6XKI	2.87 Å	A	PDB
Rabbit *(Oryctolagus cuniculus)* eEF1A2 protein structure			
4C0S	2.70Å	A/B	PDB
Human (*Homo sapiens*) Ornithine Decarboxylase protein structure			
1D7K	2.10 Å	A/B	PDB

From the retrieved structure 6XKI (*Mus musculus*) and 5ZC9 (*Homo sapiens*) were selected as they were having similar A chain and bound to RNA and 5ZC9 had 2.00Å and 6XKI had 2.87Å resolutions which were similar compared to other structures. They also were previously bound to drugs- 6XKI was bound to DMPatA and 5ZC9 was bound to Rocaglamide drug (15) at the same region of target protein and for eEF1A2 protein of rabbit (4C0S) was selected as it was only available structure among other animal models. For other animal models, from previously studied research papers (4)(6) the residues which bind to the drug were obtained. They were analysed for conservation in MSA. For Ornithine Decarboxylase protein of humans (1D7K) was selected as the only structure available in PDB format in remaining animals models for structural interaction 1D7K was considered as it was having identical drug binding residue. FoldX

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(FoldX5) was used to mutate the drug binding amino acids, which are different from animal models compared to humans to know the drug-protein interaction in specific animal models.

2.4 Protein docking process and interaction studies

In order to get functional insights and to evaluate the interaction between eIF4A1 and drug DMPatA, docking analysis between eIF4A1 proteins of selected animal models (Table S1) and drug DMPatA (PubChem Compound ID 10053416) (https://pubchem.ncbi.nlm.nih.gov) was performed using Autodock Vina features in UCSF Chimera (Chimera-1.15). The docking results were analysed using the pyMOL and ChimeraX (ChimeraX 1.2.5). PLIP tool (Protein Ligand Interaction Profiler) was used to know about the bonding between drug DMPatA and eIF4A1.

2.5 Downstream effects

For further analysis of drug action similarity in human and animal models, the effects and downstream processes of the protein were studied in the VEGF and FAS pathway (6). Sequence similarity was checked for all the proteins involved in the downstream effects to know whether the effect of the drug action will remain the same in the downstream pathway.

2.6 Structure visualizations

UCSF ChimeraX, UCSF Chimera and PyMOL were used for structural visualization and model depiction.

3. RESULT AND DISCUSSION

3.1 Retrieval of Sequences

The anticancer drug DMPatA bind to their target proteins eIF4A1 (Eukaryotic initiation factor 4A-I) which is a ATP-dependent RNA helicase, Plitidepsin which binds to eEF1A2 (Elongation factor 1-alpha 2) which is a GTP-dependent binding of aminoacyl-tRNAs to the A-site of Ribosome. The drug Geneticin binds to the Ornithine Decarboxylase which catalyzes the first step of protein synthesis. To know whether the interaction of the drug in animals model as compared to their human counterpart are the same or different, the eIF4A1, eEF1A2 and Ornithine Decarboxylase proteins sequences of various animal models were retrieved (Table S1,S2,S3 supplementary section) and analysed to find any pattern of conservation in eIF4A1, eEF1A2 and Ornithine Decarboxylase protein**.**

3.2 Sequence similarity by sequence alignment

The Multiple Sequence Alignment (Fig.2) between human and the animal models - monkey, rabbit, mouse, rat, frog, and zebrafish showed 99.75%, 100%, 100%, 100%, 95.75%, and 89.66% respectively. The drug DMPatA binds to the target eIF4A1 protein and RNA. The drug DMPatA binds to the protein residues Arg282, Asp198, Phe163, Asn167 and the residues Arg282, Gly304 and Asp305 also binds with RNA segments which were known earlier(3). These drug binding residues in all the animals the drug binding residue were identical except for zebrafish in which the Asp198 is replaced by Glu198 (shown in green colour).

The Multiple Sequence Alignment of human and animal models and the percent identity matrix shows that monkey and rabbit sequences of eEF1A2 which is bound by the drug Plitidepsin His295, Leu77, Arg37, Ser291, Tyr254, Ile256, Ile259, Val262, Trp78, Leu63 and Thr38 (4) show 100% similarity, whereas mouse and rat sequences show 99.78%, the frog 98.49% and zebrafish shows 94.17% similarity and for the protein Ornithine decarboxylase and the percentage identity matrix (Fig.4) of the protein sequence of the human and animal models shows that highest similarity is seen with monkey that is 98.05% followed by rat , mouse and frog of 91.54% , 90.57% and 80.83 % respectively and zebrafish shows the least similarity of 74.51% . The amino acids and their position which binds to the drugs are highlighted in the (Fig.4) which are Phe397, Asn398 and Phe400 (5). The drug binding amino acids on the protein are identical and conserved among the animal model organisms.

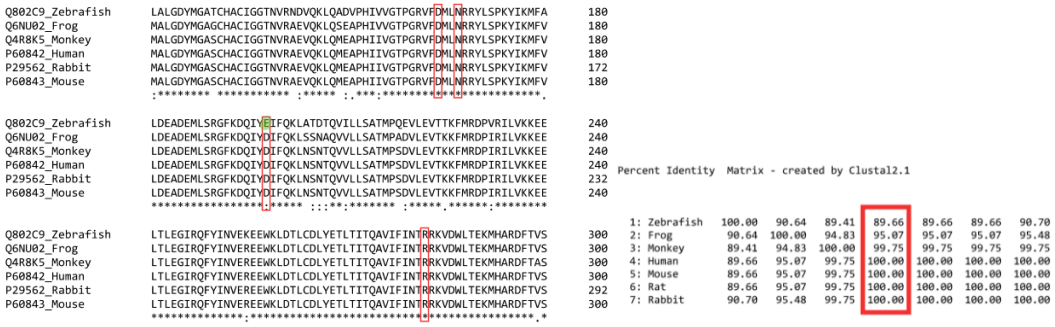

Fig 2. Multiple sequence alignment of the protein eIF4A1.The drug DMPatA binding residues are highlighted in red and the variant residue is highlighted in green. Percentage matrix of the alignment. The highlighted region in the red box shows the percentage similarity of Animal models with humans.

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Fig 3. the amino acid residues in protein eEF1A2 interacting with drug Plitidepsin in human and animal models and percentage matrix of the alignment. The highlighted region in the red box shows the percentage similarity of Animal models with humans.

Fig 4. Multiple sequence alignment of the protein Ornithine Decarboxylase among human and animal models. The similarity percentage of sequence of the protein Ornithine Decarboxylase among human and animal models, the highlighted region in the red box shows the percentage similarity of Animal models with humans.

3.3 Structure templates searching and structural mutation.

From the retrieved structures of drug bound target proteins from Uniprot and pdb, the proteins were extracted from drugs and aligned to check the structural similarity between human and animal models. Mouse eIF4A1 was superimposed (Fig. 5C) on Human eIF4A1 counterpart. The RMSD value between the superimposed structures is 0.394 angstroms. The other animal models protein structures were also similar to the mouse eIF4A1, except for zebrafish RMSD value is 0.897 angstroms. To study the interaction the residue Aspartic acid 198 was mutated to Glutamic acid 198, the mouse eIF4A1 and zebrafish eIF4A1 are superimposed to see the residual change in the structure (fig.5 D).The retrieved structures from Uniprot and pdb was only for human (Fig.5A), the drug binding amino acids of animal models protein structures were also identical to the human Ornithine Decarboxylase, To study the interaction the drug binding residues were identified in the structure based on their number. For other proteins the structures retrieved were rabbit eEF1A2 (4C0S) and human Ornithine Decarboxylase (1D7K).

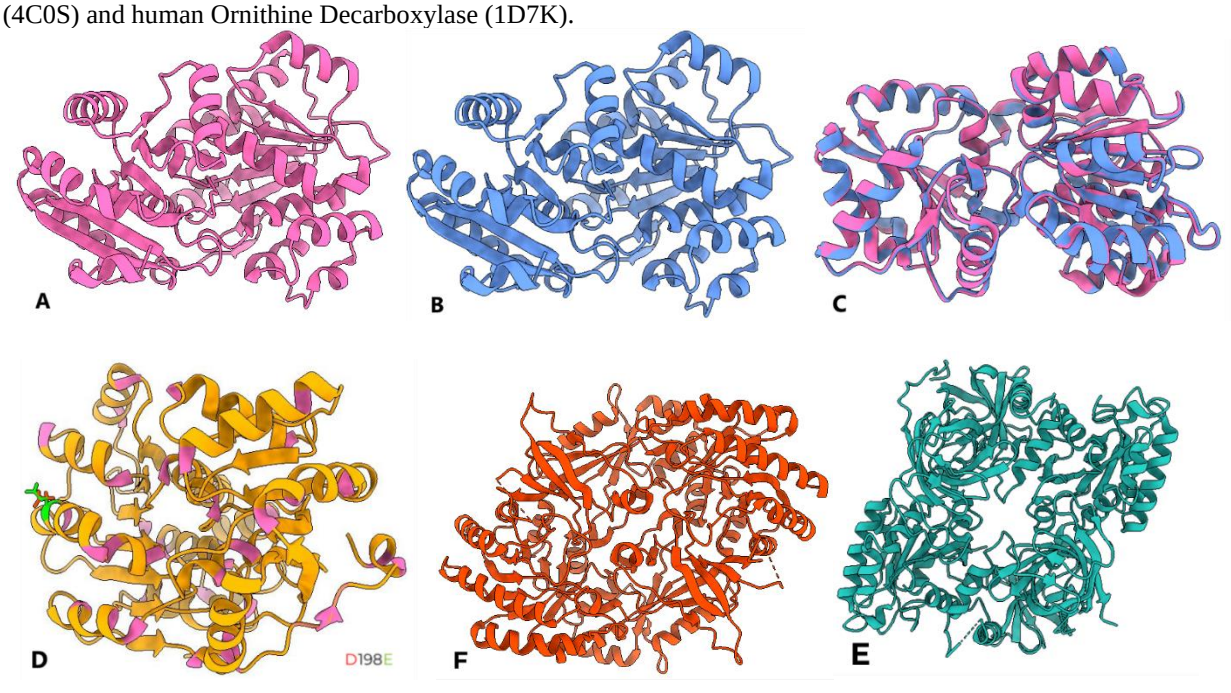

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Fig.5. A. protein structure of human eIF4A1 (5ZC9), B. protein structure of mouse eIF4A1 (6XKI), C. Superimposition of the human and mouse eIF4A1. D. the Mutant model built from FoldX are superimposed with human eIF4A1. The residue D (Asp198) highlighted in red and mutated E (Glu198) are highlighted in green. Visualized in ChimeraX. E. protein structure of Rabbit eEF1A2 (4C0S), F. protein structure of Human Ornithine Decarboxylase (1D7K).

3.4 Protein docking process and interaction studies

The target proteins eIF4A1 were docked with drug DMPatA in Autodock Vina and visualized in Chimera. Interactions were studied for knowing the bonding in human, mouse and zebrafish (FoldX mutated model). The amino acids Arg282 bonds by Water Bridge, Asp197 bond by hydrogen bond, Phe163, Asn163 bond by hydrophobic interactions. For Plitidepsin and Geneticin the drug was docked to their target protein to know the interactions of the drug and their target protein were bound with only hydrophobic interactions and hydrogen bonds. For Plitidepsin and Geneticin drug structures were not available hence the interactions were not predictable.

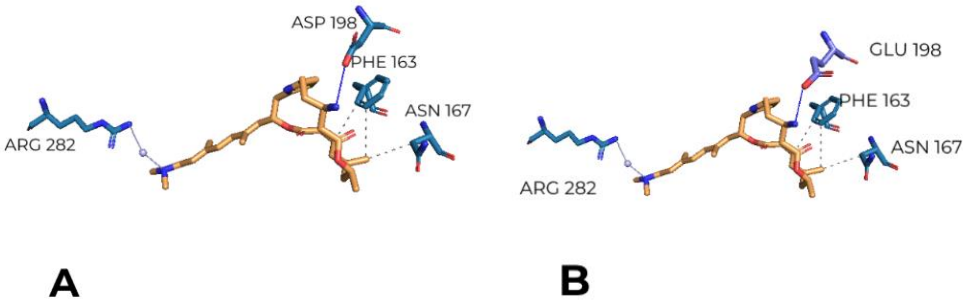

Fig.6. Images showing the docking results of the drug DMPatA and eIF4A1. A. images showing the bonding of drug and protein in mice. B. the bonding activity of the drug and protein in zebrafish with mutated residue Glutamic Acid.

3.6 Downstream effects

For the drug DMPatA binding to protein eIF4A1, there are no downstream proteins as the drug directly inhibits RNA translation by strongly attaching over the RNA, not allowing translation and it also blocks the reuse of eIF4A1. The downstream effects of Plitidepsin with other protein (FAS, VEGF-R) along with target protein eEF1A2 was studied In which Plitidepsin induced apoptosis via activation of the FAS pathway, subsequent activation and release of cytochrome c from mitochondria. Plitidepsin inhibits secretion of VEGF-R by acting on VEGF not leading to cell growth. Plitidepsin inhibits secretion of VEGF-R by acting on VEGF**.** Activation of tyrosine kinase receptor by VEGF (6), whereas the phosphorylation of tyrosine residues are from tyrosine kinase.

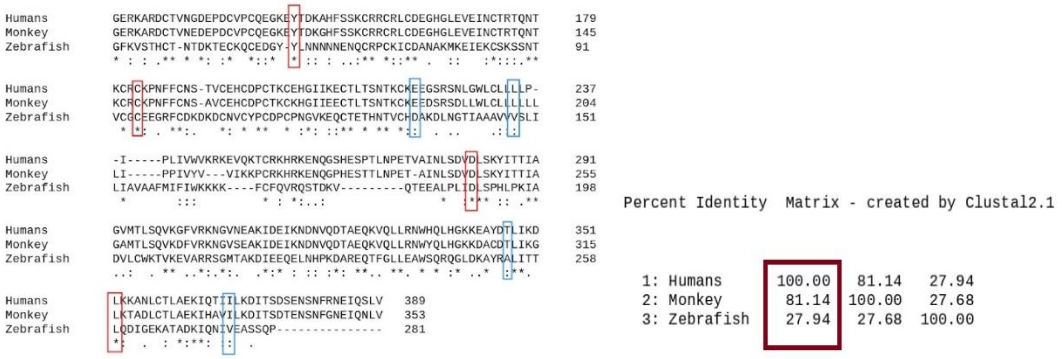

Fig 7. The FAS protein sequences showing amino acids marked in red are conserved whereas amino acids highlighted in blue are similarly replaced in zebrafish and the percent identity matrix showed more similarity percentage with monkey 81.14% than zebrafish 27.94%.

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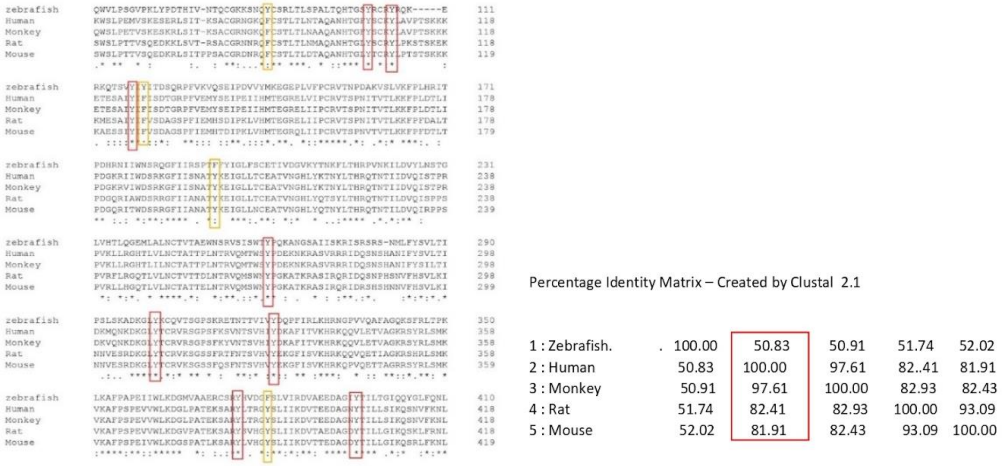

Fig 8. Multiple sequence alignment shows the tyrosine residues are conserved which are marked in red and tyrosine are similarly replaced with phenylalanine marked in yellow. Based on percent identity, the matrix shows monkeys having 97% similarity, rats 82%, mice 81% and zebrafish 50%.

DISCUSSION:

Cancer is one of the most prevalent among the fatal diseases. New antitumor and anti-cancer drugs are being tested in human animal models before testing in preclinical studies. But these studies are severely restricted by animal testing regulations and availability of suitable animal models. It becomes important therefore to select the right animal model whose biology closely mimics that of humans at least in the drug binding and downstream effects.

In this work by computational analysis, modelling and docking studies we show through *in-silico* analysis of which animal models are suitable for testing specific cancer drugs based on their interactions with their target proteins. The analysis was in three drugs DMPatA with target protein eIF4A1, Plitidepsin with target protein eEF1A2 and Geneticin with target protein Ornithine decarboxylases. For studying the effectiveness and interaction in human and animal models we selected monkey, rabbit, mouse, rat, frog and zebrafish all having proper drug binding residue. Since this investigation is done *in-silico* we suggest that these results can only at best inform the animal testing choices and cannot substitute for *in-vivo* studies. This allows us to select the suitable animal models. By this study researchers can pre analyze the suitable animal models subjects which makes the research into animal model testing more effective.

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Supplementary section:
https://docs.google.com/document/d/1YHPaSXu_-oArvHwgLySu12ssBBOSwMAFKaB2i9SGAYc/edit