RESEARCHS / INVESTIGACIÓN

Polypharmacological study of Ceritinib using a structure based *in silico* approach

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Abstract: Drug repurposing has gained mass recognition over the past few years as it has paved new therapeutic applications for already approved FDA drugs. It focuses on finding new molecular targets of drugs for medical uses different than the one originally proposed. Ceritinib, an Anaplastic Lymphoma Kinase (ALK) inhibitor is given orally in the treatment of non-small cell lung cancer (NSCLC). This treatment has been reported to be associated with a number of side effects such as hyperglycemia, convulsion, pneumonitis etc. The side effects are usually due to the unintended interaction of the drug with other protein targets. *In silico* polypharmacological studies of Ceritinib suggests that it binds to multiple targets other than the intended one which may largely be due to different proteins possessing similar binding sites. ProBis server was used to retrieve probable off-targets of Ceritinib based on presence of structurally similar protein binding sites as that of ALK. Ceritinib was found to bind effectively to three proteins namely Lymphocyte Cell-Specific Protein-Tyrosine Kinase, Tropomyosin receptor kinase B and Aurora kinase B having favorable binding energies and inhibition constants, with no reported side-effects as compared to their marketed drugs. Therefore, it is concluded from the present study that Ceritinib may act as an effective therapeutic target against its polypharmacological targets.

Keywords: Polypharmacology, repurposing, molecular docking, protein binding similarity search.

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Introduction

Conventional drug discovery processes require huge time and a hefty amount of money to bring a single drug into the market. Hence, in silico methods play a vital role in drug discovery and development by offering an enhanced efficiency to the pharmaceutical industry[1]. Over the last four decades, the drug manufacturing companies have shifted their focus from the reductionist approach towards 'one drug, multiple targets' also known as polypharmacology. Polypharmacology has been defined as the ability of small molecules to interact simultaneously and specifically with multiple targets[2]. Polypharmacology can be broadly characterized into two types: adverse polypharmacology (which involves off–target binding) and therapeutic polypharmacology (which involves repurposing of drugs i.e. the process of searching new therapeutic applications for existing drugs also known as drug repositioning)[3].

Polypharmacology thus offers to be a promising approach for identifying novel therapeutic uses for already known drugs against complex diseases. It has been previously reported that effect of a drug on an unintended target might suggest new uses for an existing drug (i.e. multi-target drugs)[4]. Moreover, drugs which failed to provide the intended benefit can also be considered for repurposing for the treatment of other diseases. A classic example is the anti-cancer drug Zidovudine which failed in intended treatment of cancer however it was found to be effective against HIV and in 1987 was approved by FDA for HIV treatment[5].

Polypharmacological drugs are expected to have relatively lower chances for developing drug resistance as they target multiple drug targets and are considered to show better efficacy than drugs for a single target[6,7]. Several drugs have already been successfully repurposed for new diseases, outside the original medical scope. For example, the blockbuster drug Sildenafil (Viagra), a phosphodiesterase (PDE) inhibitor was first developed for treating hypertension and ischemic heart disease. During the phase I clinical trials, Sildenafil was found to induce penile erections as a side effect and after phase II clinical trial failure, sildenafil was repositioned for the treatment of erectile dysfunction and approved by the FDA[8].

Ceritinib (formerly known as LDK378 and marketed as Zykadia™), an inhibitor of Anaplastic lymphoma kinase is used to treat non-small cell lung cancer (NSCLC). It acts to inhibit the autophosphorylation of Anaplastic Lymphoma Kinase (ALK) and its downstream signaling protein STAT3 thereby preventing the proliferation of ALK-dependent cancer cells[9].

In silico computational methods are of high biological and pharmacological value as they predict new targets for existing small-molecule compounds[2]. In our previous in silico polypharmacological study, it was found that hyperglycemia caused in patients after Ceritinib treatment could be due to the off-target binding of Ceritinib to dual specificity protein kinase CDC Like Kinase 2 (CLK2) (PDB ID: 3NR9) along with its actual target Anaplastic Lymphoma Kinase[10]. Therefore, in the present study, we further investigated the polypharmacological-targets of ceritinib to identify its probable repurposing potential.

Materials and methods

Data mining of target protein of Ceritinib

The Drugs and Drug Targets mapping tool of the Protein Data Bank (PDB) was utilized to obtain the crystal structure of the target protein Anaplastic lymphoma tyrosine kinase (ALK) bound with Ceritinib (PDB ID: 4MKC)[11].

Identification of proteins with similar binding sites

The ProBis server was used to detect structurally similar protein binding sites in ALK tyrosine kinase (PDB ID: 4MKC). ProBis employs a fast maximum clique algorithm to detect similar protein binding sites independent of the sequence or the fold present in proteins[12]. Therefore, using ProBis proteins having similar geometrical and physicochemical properties to Ceritinib target ALK were retrieved.

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Docking studies of Ceritinib with probable polypharmacological/off-targets

To investigate the binding affinity of Ceritinib with proteins other than its actual target ALK tyrosine kinase, Autodock tool was used to perform drug-protein docking[13]. The binding energies and inhibition constants of polypharmacological-targets with Ceritinib and their bound ligands were tabulated and compared.

Literature mining

The probable polypharmacological-targets of Ceritinib were studied for their role in disease pathways and therapeutics in order to predict its repurposing prospects.

Results and discussion

Repurposing studies involve investigating multiple targets of drugs to enhance their biological activities as well as reduce their toxicities. In order to find polypharmacological targets of Ceritinib, the drug was docked with 33 probable off-targets obtained from ProBis server. Literature studies also revealed that these polypharmacological targets may be critical therapeutic targets in various other complex diseases such as breast cancer, Acute Lymphoblastic Leukemia (ALL) and CNS disorders. Sahrawat and Kaur (2018) while investigating the polypharmacology of Certinib have reported that proteins having similar binding sites to the actual target of Certinib i.e. ALK , retrieved from ProBis server, namely Ribosomal protein S6 kinase alpha-1 (RSK1) (PDB ID: 2Z7R), Lymphocyte Cell-Specific Protein-Tyrosine Kinase (LCK) (PDB ID: 1QPC), Tropomyosin receptor kinase B (TRKB) (PDB ID: 4AT5) and Aurora kinase B (AURKB) (PDB ID: 4AF3) had a significant Z-score > 2.0. Z-score is the measure of similarity between two protein binding sites[12] due to which different proteins may interact with same drug molecules. Further, the docking analysis of these proteins with the drug Ceritinib, revealed binding energies and inhibition constants comparable to the actual target i.e. ALK, with no reported side-effects on binding to Ceritinib[10]. Therefore, the proteins which were identified as off-targets of Ceritinib, were further investigated for repurposing of Ceritinib, so as to identify other probable usage of the drug.

The results obtained from docking server, Autodock is in terms of binding energy and inhibition constant (K_i) at temperature 298.15 K. Binding energy is the affinity of the ligand with which it binds to the target protein. More negative binding energy, higher is the affinity of the ligand to bind the receptor protein. Inhibition constant of a drug is the concentration required to produce half maximum inhibition on binding the receptor, therefore, lower the inhibition constant value lesser is the amount of drug concentration needed to produce an effect[13].

The docking studies indicated that the binding energy of Ceritinib with its actual target, ALK (PDB ID: 4MKC) was -5.62 kcal/mol with an inhibition constant of 75.9 μM μM. In addition, the docking studies of Ceritinib with proteins Ribosomal protein S6 kinase alpha-1 (RSK1) (PDB ID: 2Z7R), Lymphocyte Cell-Specific Protein-Tyrosine Kinase (LCK) (PDB ID: 1QPC), Tropomyosin receptor kinase B (TRKB) (PDB ID: 4AT5) and Aurora kinase B (AURKB) (PDB ID: 4AF3) revealed that they had higher binding affinities and lower inhibition constant for Ceritinib than its actual target ALK (PDB ID: 4KMC)[10] as shown in Table 1.

Ribosomal protein S6 Kinase alpha-1 (PDB ID: 2Z7R) retrieved from ProBis server had binding energy of -7.83 kcal/mol with Ceritinib while its marketed therapeutic agent Staurosporine (Drugbank ID: DB02010) had a higher binding energy of -10.3 kcal/mol. Further, RSK1 had a favorable inhibition constant for Staurosoporine, which has been used for treating triple negative breast cancer[14,15] (Table 1). Our results are also supported by the report of Kuenzi et al. (2017) in an integrated functional proteomics study for repurposing of the anaplastic lymphoma kinase (ALK) inhibitor Ceritinib, wherein it was reported that IGF1R, RSK1, RSK2 and FAK1 are potential off-targets of Ceritinib[16].

LCK is a potential target in the treatment of T-cell Acute Lymphoblastic Leukemia (ALL) and its marketed drug is Dasatinib (D number: D03658)[17, 18]. Moreover, for the treatment of Central nervous system (CNS) disorders and various tumors including NSCLC, TRKB has been used as a potential therapeutic target and Entrectinib (D number: D10926) is prescribed for its treatment[19,20]. The probable polypharmacological targets of Ceritinib, namely LCK protein (PDB ID: 1QPC) and TRKB (PDB ID: 4AT5) had higher binding affinity and lower inhibition constant for Ceritinib as compared to their marketed therapeutic agents Dasatinib and Entrectinib, respectively (Table 1).

S.No.	Protein	Drug*	PDB ID	Binding energy (kcal/mol)		Inhibition constant (μM)	
				Ceritinib	Drug*	Ceritinib	Drug*
1.	RSK1	Staurosporine	2Z7R	-7.83	-10.30	1.83	0.02
2.	LCK	Dasatinib	1QPC	-7.49	-6.25	3.23	26.42
3.	TRKB	Entrectinib	4AT5	-7.43	-6.32	3.59	23.11
4.	AURKB	Tozasertib	4AF3	-7.18	-7.64	5.45	2.50

Table 1: Binding energy and inhibition constant values of polypharmacological-targets with Ceritinib and their reported therapeutic agents.

On docking of Aurora kinase B (PDB ID: 4AF3) with its marketed therapeutic agent Tozasertib (D number: D08279)[21], it was found that there was not much difference in their binding energies, although the inhibition constant was higher for Ceritinib. AURKB has been reported to be involved in various cancers such as breast cancer, hepatocellular carcinoma, lung adenocarcinoma, colorectal cancer models and acute lymphoblastic leukemia (ALL) which are currently being treated using Tozasertib[22,23].

On investigation of binding site residues of polypharmacological-targets of Ceritinib it was seen that all the proteins RSK1, LCK, TRKB and AURKB had similar binding site interacting residues as that of Ceritinib with its actual target ALK (PDB ID: 4KMC) tabulated in Table 2, Figure 1.

Analysis of the docking scores of all the four poypharmacological

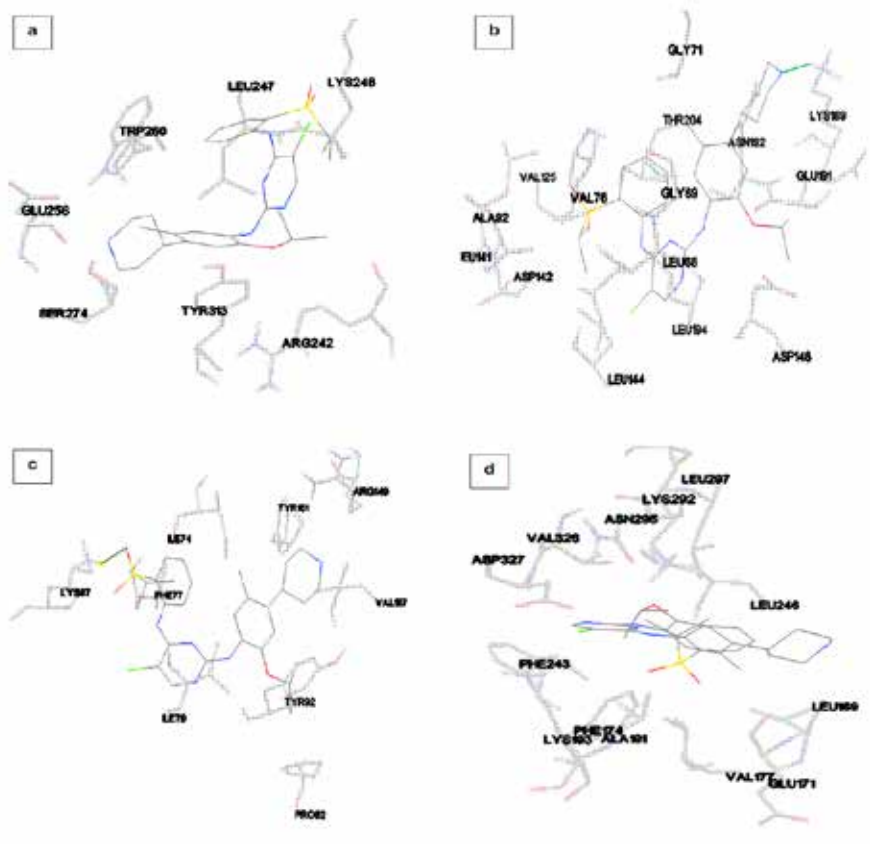

Figure 1: Interacting amino acid residues in the binding pockets of a) Ribosomal protein S6 Kinase alpha 1 (PDB ID: 2Z7R) b) LCK (PDB ID: 1QPC) c) TRKB/BDNF/NT-3 growth factors receptor (PDB ID: 4AT5) d) Aurora Kinase B (PDB ID: 4AF3) docked with Ceritinib visualized using AutoDock analysis tool.

S. No.	Protein	PDB ID	Interacting residues
1.	ALK	4KMC	LEU1122, VAL1130, LYS1150, GLU1167, ILE1171, PHE1174, ILE1179, MET1199, ASP1203, SER1206, GLU1210, LEU1256, GLY1269, ASP1270, PHE1271
2.	RSK1	2Z7R	LEU68, GLY69, GLY71, VAL76, ALA92, VAL125, LEU141, ASP142, LEU144, ASP148, LYS189, GLU191, ASN192, LEU194, THR204
3.	LCK	1QPC	ARG242, LYS246, LEU247, GLU258, TRP260, SER274, TYR313
4.	TRKB	4AT5	ALA564, PHE565, ASP600, GLU604, LEU607, PHE688, ARG691, ASP710, GLY712
5.	AURKB	4AF3	ILE74, PHE77, ILE79, PRO82, TYR92, LYS97, VAL107, ARG149, TYR151

Table 2: Binding site residues of actual target and polypharmacological-targets of Ceritinib.

targets namely Ribosomal protein S6 kinase alpha-1, LCK, Aurora kinase B and (Table 2) indicated that Ceritinib binds effectively to three proteins namely LCK, AURKB and TRKB as compared to their available marketed drugs.

Conclusion

Therefore, it may be concluded that three polypharmacological targets of Ceritinib i.e LCK, Aurora kinase B and Tropomyosin receptor kinase B, identified in the present study, bind to the

Ceritinib more effectively, as seen from their high binding energies, as compared to the drug's actual target ALK. This study paves new opportunities for Ceritinib in the treatment of various complex diseases such as cancer, central nervous disorders etc. associated with these proteins. In addition, experimental studies need to be carried out to further validate the results of this work.

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