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Investigation of Off-targets of NSCLC drug Ceritinib: A Structure Based Systems Biology Approach

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Abstract

Drugs used in cancer chemotherapy usually have a narrow therapeutic index and often the responses produced include various side effects. Ceritinib, an Anaplastic Lymphoma Kinase receptor inhibitor, is one such drug that is used in the treatment of non-small cell lung cancer (NSCLC). However, this drug has been reported to have certain side effects and complications. Therefore, this in silico study focuses on the identification of off-targets of Ceritinib using a structure based systems biology approach. The structure of ALK-Ceritinib complex (PDB ID: 4MKC) was used for identification of similar protein binding sites using ProBis which is a structure based method. The putative off-targets were then studied using molecular docking. In the present study we identified an off-target of Ceritinib namely Cdc2-like kinase (CLK2) that may be responsible for the reported side effect of hyperglycemia.

Keywords: *Polypharmacology, lung cancer, off-targets, protein binding similarity search, molecular docking.*

1. Introduction

Computational methods play a crucial part in drug discovery and development [1]. They are expected to reduce the cost of drug discovery process by limiting chemical synthesis and biological testing. New drugs that successfully enter the market after clinical trials may still be withdrawn due to their unexpected side effects which may occur due to off-target binding of drugs and the lack of systems-level understanding of drug response. Over the last four decades, the drug manufacturing companies have shifted their focus from the reductionist approach to a new paradigm called polypharmacology *i.e.* 'one drug, multiple targets' [2]. Polypharmacology can be further divided into two types: adverse polypharmacology (which involves off–target binding) and therapeutic polypharmacology (which involves repurposing) [3].

One of the leading causes of cancer death amongst men and women is lung cancer [4]. The exposure of lungs to the external environment is a contributing factor responsible for lung cancer with smoking being one of its most common cause. Lung cancer has two main types namely small-cell lung cancer and non small-cell lung cancer. Small-cell lung cancer also known as oat cell cancer accounts for about 10-15% of lung cancer and is different from the other type because of its clinical and biological features being extremely sensitive to radiation and chemotherapeutic agents [5]. Non small-cell lung cancer (NSCLC) represents more than 85% of lung cancer cases and is often described as a group of discrete diseases having genetic and cellular heterogeneity. The chromosomal rearrangements of Anaplastic lymphoma kinase (ALK) have been reported in 3% to 7% of non–small cell lung cancers [6]. In recent years, the design of chemotherapeutics is directed against cancer specific molecules. Tyrosine kinase is one such family of enzymes that has been implicated in the pathophysiology of cancer [7, 8]. These enzymes catalyze phosphorylation of selected residues in target protein using ATP which has a pivotal role in cellular homeostasis and any aberrations in its activity may cause neoplastic development and progression. ALK is a type-I transmembrane tyrosine kinase - a member of insulin receptor superfamily which plays a vital role in the early development and maintenance of the central nervous system. The ALK gene may become oncogenic by forming a fusion gene with EML4 which autophosphorylates its intracellular ALK kinase domain and consequently activates RAS signaling pathway thereby resulting in uncontrolled cell growth [9].

ALK tyrosine kinase inhibitors (TKIs) have been developed as antitumor therapeutic agents in treating NSCLC [10] and Ceritinib is one such drug. Ceritinib acts by inhibiting the autophosphorylation of ALK by further preventing ALK-mediated phosphorylation of the downstream signaling protein

STAT3, and proliferation of ALK-dependent cancer cells, thereby preventing the onset of cancer [11].

However, some adverse effects have been reported following Ceritinib treatment such as convulsion, pneumonitis, dehydration, hyperglycemia, and nausea [12]. It is hypothesized that these adverse side-effects may be due to the binding of Ceritinib to its polypharmacological targets other than the actual target. Therefore, the present study was undertaken to identify the possible off-targets for the drug Ceritinib using a structure based systems biology approach.

2. Methods

2.1 Retrieval of target protein for Ceritinib

The crystal structure of the target protein Anaplastic Lymphoma receptor tyrosine kinase (ALK tyrosine kinase) of Ceritinib was searched using Drug and Drug target mapping tool of PDB. The crystal structure of the target with bound Ceritinib was retrieved from PDB having PDB ID: 4MKC [13].

2.2 Identification of potential off-targets of Ceritinib

ProBiS Server (http://probis.cmm.ki.si/) was used to identifyother proteins having binding sites similar to ALK tyrosine kinase (PDB ID: 4MKC) and uses clique detection method. Structures with surface regions having similar geometrical and physicochemical properties to those in the query structure were retrieved [14].

2.3 Protein-ligand docking

Docking of the NSCLC drug Ceritinib with its probable off-target proteins was performed using Autodock tool (version 4.2.6) (http://autodock.scripps.edu/) that uses the Lamarckian Genetic Algorithm and empirical free energy scoring function [15].

3. Results and Discussion

The crystal structure of Anaplastic lymphoma receptor tyrosine kinase (ALK) with bound Ceritinib was retrieved from PDB having PDB ID: 4MKC [13]. ProBis server identified 288 proteins with similar binding sites to ALK and ranked them according to their Z-scores. Z-score is the measure of similarity between two protein binding sites [14]. In this study, two binding sites were defined as similar if their similarity Z-score was higher than 2.0. The proteins retrieved from ProBis could be the probable off-targets of Ceritinib. From the proteins obtained, 33 proteins that are found in *Homo sapiens* (Table 1) were further investigated by docking with Ceritinib using the Autodock tool.

The docking results of Ceritinib with its probable off-targets were compared with the results of its actual target ALK (PDB ID: 4MKC) that had a binding energy of -5.62 kcal/mol and inhibition constant value of 75.91μM. The scores obtained from AutoDock were ranked according to their binding energies (Table 2). The protein ranked 1 in Table 2 was excluded from further study due its positive binding energy. Similarly, the proteins ranked 3-8 in Table 2 were also rejected on the basis of their high inhibition constant values since higher values of inhibition constant signifies that higher concentration of drug is needed to produce maximum inhibition which is unfavorable in terms of dose exposure to humans [15]. The proteins ranked 26-33 in Table 2 had binding energy less negative than -5.62 kcal/mol and were not further considered as they would be unable to bind Ceritinib effectively. The proteins ranked 2 and 9-25 from Table 2 were therefore considered for investigating the probable off-targets of anti-cancer drug Ceritinib to understand its polypharmacology effects.

All these proteins identified by the ProBis server have Lysine as a common ATP binding site is similar to the actual target ALK tyrosine kinase. The interacting residues of ATP binding pocket of CLK2 and ALK were similar (Figure 1) having amino acid residues Leucine, Glutamate, Phenyalanine, Valine, Lysine and Aspartate. Docking results further revealed that the protein dual specificity protein kinase Cdc2 like kinase (CLK2) with PDB ID: 3NR9_A binds Ceritinib most efficiently with lowest negative binding energy value of -10.22 kcal/mol and an inhibiton constant of 32.51 μM which was lower than ALK.

One of the reported side effects of Ceritinib treatment is hyperglycemia that is characterized by excessive amount of glucose circulation in the blood [16]. Studies have identified that CLK2 acts as a component of hepatic insulin signaling and glucose metabolism and is an insulin regulated suppressor of gluconeogenesis and glucose output. Rodgers *et.al* in 2010 have proposed a model to explain the mechanism of autophosphorylation of CLK2 protein which enhances its stability.

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Table 1: Off-targets of Ceritinib for *Homo sapiens* obtained from ProBis server

S.no.	PDB Chain	Protein	Z-score*
1.	3BKB_A	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE FES/FPS	2.86
2.	4PMP_A	HIGH AFFINITY NERVE GROWTH FACTOR RECEPTOR	2.83
3.	4AT5_A	BDNF/NT-3 GROWTH FACTORS RECEPTOR	2.78
4.	1MQB_A	EPHRIN TYPE-A RECEPTOR 2	2.72
5.	3ZBF_A	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE ROS	2.71
6.	1QPC_A	LCK KINASE	2.68
7.	4HZR_A	ACTIVATED CDC42 KINASE 1	2.64
8.	1P4O_A	INSULIN-LIKE GROWTH FACTOR I RECEPTOR PROTEIN	2.63
9.	3LCD_A	MACROPHAGE COLONY-STIMULATING FACTOR 1 RECEPTOR	2.62
10.	2VWX_A	EPHRIN TYPE-B RECEPTOR 4	2.59
11.	2HK5_A	TYROSINE-PROTEIN KINASE HCK	2.59
12.	4U6R_A	SERINE/THREONINE-PROTEIN KINASE/ENDORIBONUCLEASE IRE1	2.53
13.	3LXP_A	NON-RECEPTOR TYROSINE-PROTEIN KINASE TYK2	2.49
14.	3B2T_A	FIBROBLAST GROWTH FACTOR RECEPTOR 2	2.46
15.	3NR9_A	DUAL SPECIFICITY PROTEIN KINASE CLK2	2.4
16	3C1X_A	HEPATOCYTE GROWTH FACTOR RECEPTOR	2.39
17.	4I4E_A	FOCAL ADHESION KINASE 1	2.37
18.	1RJB_A	FL CYTOKINE RECEPTOR	2.34
19.	4TWC_A	CASEIN KINASE I ISOFORM DELTA	2.31
20.	3QD2_B	EUKARYOTIC TRANSLATION INITIATION FACTOR 2-ALPHA KINASE 3	2.3
21.	4AF3_A	AURORA KINASE B	2.27
22.	4NST_A	CYCLIN-DEPENDENT KINASE 12	2.23
23.	3ALO_A	DUAL SPECIFICITY MITOGEN-ACTIVATED PROTEIN KINASE KINASE4	2.23
24.	2I6L_A	MITOGEN-ACTIVATED PROTEIN KINASE 6	2.22
25.	3M2W_A	MAP KINASE-ACTIVATED PROTEIN KINASE 2	2.21
26.	3PP0_A	RECEPTOR TYROSINE-PROTEIN KINASE ERBB-2	2.21
27.	2Z7R_A	RIBOSOMAL PROTEIN S6 KINASE ALPHA-1	2.21
28.	4ASE_A	VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2	2.21
29.	2Y7J_A	PHOSPHORYLASE B KINASE GAMMA CATALYTIC CHAIN, TESTIS/LIVER ISOFORM	2.16
30.	1T46_A	HOMO SAPIENS V-KIT HARDY-ZUCKERMAN 4 FELINE SARCOMA VIRAL ONCOGENE HOMOLOG	2.15
31.	2W4O_A	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE IV	2.13
32.	2ACX_A	G PROTEIN-COUPLED RECEPTOR KINASE 6	2.12
33.	1QCF_A	HAEMATOPOETIC CELL KINASE (HCK)	2.11

*Z-score >2.0 indicates similar binding sites

They have reported that in response to insulin signaling through PI3K/Akt, CLK2 kinase activity is induced by phosphorylation of the activation loop residue T343. CLK2 can then phosphorylate the SR domain on PGC-1α causing repression of PGC-1α transcriptional activity on gluconeogenic genes and reducing hepatic glucose output [17].Therefore, from the structure based systems biology study it is concluded that Ceritinib may bind to CLK2 kinase during treatment regimes of NSCLC thereby inhibiting it and causing the observed hyperglycemia, the reported side-effect of Ceritinib treatment, due to increased gluconeogenesis. None of the other off-targets identified using ProBis server were found to be associated with the reported side-effects of Ceritinib.

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Table 2: Showing AutoDock results in terms of binding energy and inhibition constant

Rank	PDB ID	AutoDock Binding energy (kcal/mol)	AutoDock Inhibition constant(μM)
1.	1RJB_A	+2.68	-
2.	3NR9_A	-10.22	32.51
3.	4NST_A	-8.88	310.88
4.	2Y7J_A	-8.72	407.74
5.	3LCD_A	-8.48	607.42
6.	3M2W_A	-8.44	649.66
7.	2HK5_A	-8.38	716.37
8.	4I4E_A	-8.31	806.60
9.	2Z7R_A	-7.83	1.83
10.	1QPC_A	-7.49	3.23
11.	4AT5_A	-7.43	3.59
12.	4AF3_A	-7.18	5.45
13.	3BKB_A	-6.88	9.12
14.	2ACX_A	-6.60	14.61
15.	4HZR_A	-6.57	15.32
16.	2VWX_A	-6.48	17.90
17.	1MQB_A	-6.45	18.82
18.	4ASE_A	-6.23	27.20
19.	3C1X_A	-6.19	28.84
20.	1T46_A	-6.16	30.52
21.	3ZBF_A	-6.10	33.55
22.	3B2T_A	-6.09	34.20
23.	1QCF_A	-6.07	35.83
24.	2I6L_A	-5.94	44.52
25.	4U6R_A	-5.76	59.77
ALK	4MKC_A	-5.62	75.91
26.	4TWC_A	-5.57	83.04
27.	1P4O_A	-5.47	98.07
28.	2W4O_A	-5.27	136.21
29.	3PP0_A	-5.16	164.65
30.	4PMP_A	-5.12	177.96
31.	3QD2_B	-5.02	209.91
32.	3LXP_A	-4.72	349.45
33.	3ALO_A	-4.66	381.09

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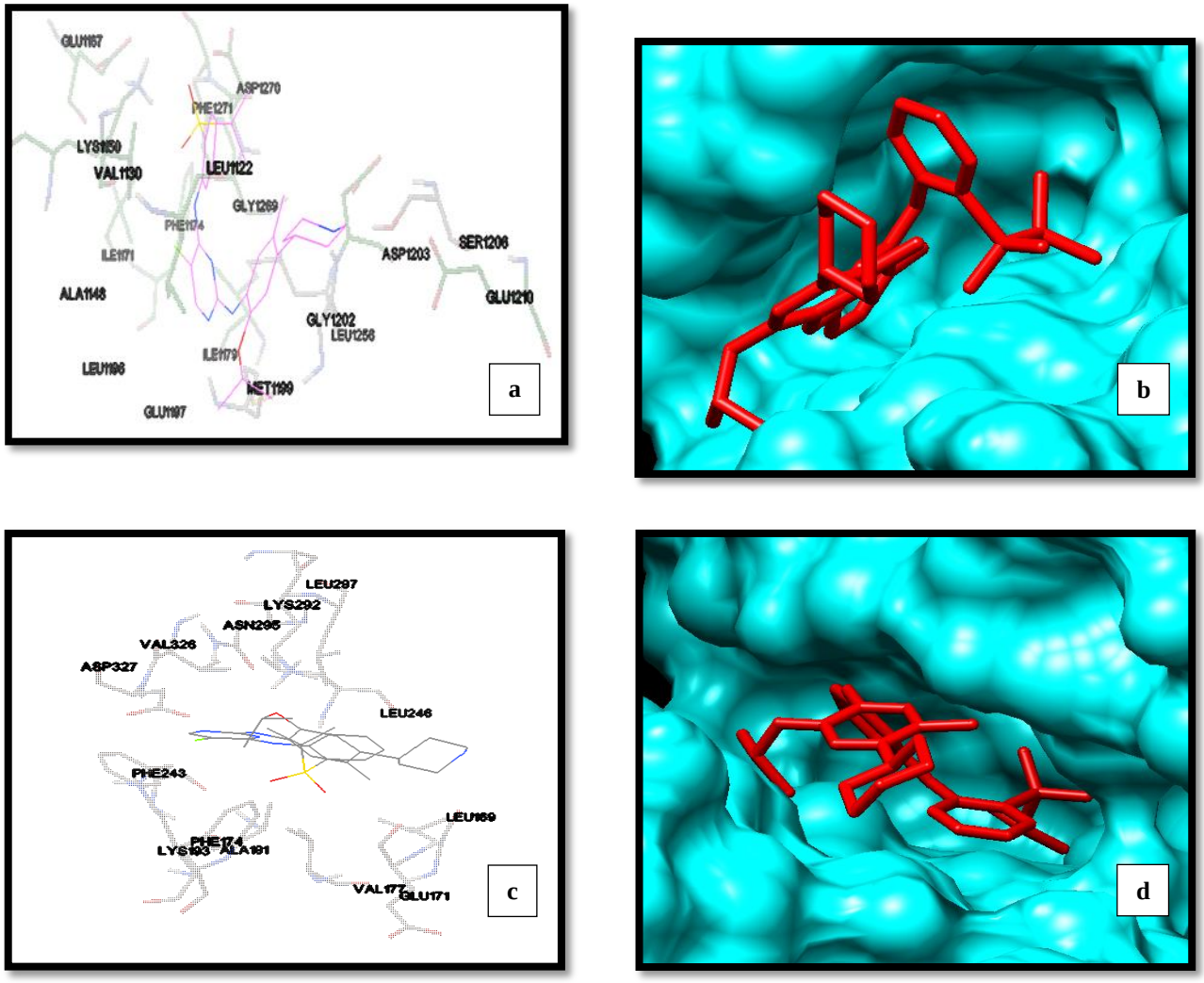

Figure 1: a) Interacting residues and b) docking of Ceritinib with ALK tyrosine kinase c) Interacting residues and d) docking of Ceritinib with CLK2; visualized using AutoDock and UCSF Chimera.

4. Conclusion

In recent times, a number of computational approaches are being applied to the study of polypharmacology for identification of possible off-targets of drugs before clinical trials to prevent costly failures. The present study was envisioned to identify off-targets of Ceritinib to account for its reported adverse side effects. From the structure based systems biology approach it was found that Ceritinib binds to CLK2 more effectively and has a lower inhibition constant than its actual target ALK. This suggests that interaction of Ceritinib with CLK2 resulting in its inhibition, may be responsible for the reported side-effect of hyperglycemia.

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