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An inverse docking approach for identifying new potential anti-cancer targets

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ABSTRACT

Inverse docking is a relatively new technique that has been used to identify potential receptor targets of small molecules. Our docking software package MDock is well suited for such an application as it is both computationally efficient, yet simultaneously shows adequate results in binding affinity predictions and enrichment tests. As a validation study, we present the first stage results of an inverse-docking study which seeks to identify potential direct targets of PRIMA-1. PRIMA-1 is well known for its ability to restore mutant p53's tumor suppressor function, leading to apoptosis in several types of cancer cells. For this reason, we believe that potential direct targets of PRIMA-1 identified *in silico* should be experimentally screened for their ability to inhibit cancer cell growth. The highest-ranked human protein of our PRIMA-1 docking results is oxidosqualene cyclase (OSC), which is part of the cholesterol synthetic pathway. The results of two followup experiments which treat OSC as a possible anti-cancer target are promising. We show that both PRIMA-1 and Ro 48-8071, a known potent OSC inhibitor, significantly reduce the viability of BT-474 and T47-D breast cancer cells relative to normal mammary cells. In addition, like PRIMA-1, we find that Ro 48-8071 results in increased binding of p53 to DNA in BT-474 cells (which express mutant p53). For the first time, Ro 48-8071 is shown as a potent agent in killing human breast cancer cells. The potential of OSC as a new target for developing anticancer therapies is worth further investigation.

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1. Introduction

Inverse docking, first proposed in 2001 by Chen and Zhi [1] refers to computationally docking a specific small molecule of interest to a library of receptor structures. The technique may be used to identify new potential biological targets of known compounds [2–4], or to identify targets for compounds among a family of related receptors [5]. The technique has shown success in distinguishing between homology models of receptors [5]. The technique may also be used to generate a compound's predicted pharmacological profile [6], or to generate a virtual selectivity profile that characterizes the promiscuity of the inhibitors [7]. Given the multi-faceted nature of a pharmacologically active compound's biological effects, inverse docking is especially helpful, because it may generate new hypotheses for the action mechanism.

Our docking software package, MDock, can be used for inverse docking, as demonstrated in the present work (zoulab.dalton.missouri.edu/software.htm). MDock uses a novel scoring function, ITScore, which was generated using an iterative method of deriving pair interaction potentials that avoids the problem of defining a specific reference state [8]. For the first time, the full energy landscape (both native and non-native modes) was considered in the potential derivation using a physics-based global iterative function. ITScore's binding pose and affinity predictions were extensively evaluated using diverse test sets prepared by other labs [8,9]. ITScore was also assessed using enrichment tests for virtual database screening against four target proteins [9]. In the present study, we test the ability of MDock on *in silico* inverse screening applications.

Specifically, we aim at searching for potential protein targets of PRIMA-1. Found from high-throughput screening, PRIMA-1 (p53 reactivation and induction of massive apoptosis, shown in Fig. 1), is a small molecule capable of activating mutant p53 protein, restoring its ability to bind to DNA and the tumor suppressor function

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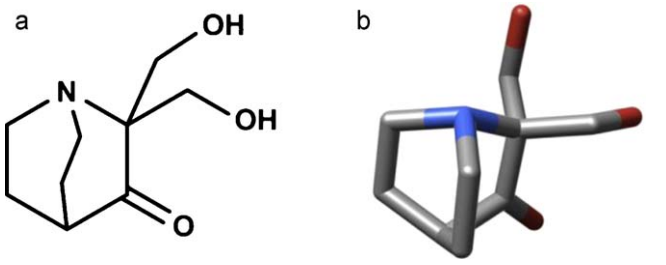

Fig. 1. (a) Chemical structure, generated using MarvinSketch 4.1.0 (www.chemaxon.com), and (b) 3D structure of PRIMA-1. Hydrogen atoms are omitted from the 3D structure for clarity.

associated with wild-type p53 [10,11]. This effect has been demonstrated *in vitro* and *in vivo*, and has been shown to trigger massive apoptosis in several types of human breast cancer cells [12,13]. PRIMA-1 is also known to stimulate expression of p21 and other p53-dependent promoters in mutant p53 breast cancer cell lines. PRIMA-1's importance as a potential agent against cancer is well-established. Nevertheless, while specific mechanisms have been proposed for PRIMA-1's mutant p53 reactivation effect [10,14,15], none have gained wide acceptance and the question remains unsettled. For this reason, we consider PRIMA-1 well suited as the subject of an inverse docking study.

In this work, we used the inverse-docking approach to screen for potential molecular targets of PRIMA-1. The objective is to guide future assays of the inhibitors of these predicted targets for their efficacy in inhibiting tumor cell proliferation, as such results may lead to potential cancer treatments, as well as provide clues regarding PRIMA-1's action mechanism. We used MDock to perform this study. In support of our approach, here we present the first stage results of our assays of Ro 48-8071, a known potent inhibitor of oxidosqualene cyclase (OSC) [16,17], the highest-ranked human protein of our *in silico* study. We show that Ro 48-8071 is a novel potent agent in selectively reducing the viability of BT-474 and T47-D cells, which are both human breast cancer cell lines that express mutant p53. In addition, we found that Ro 48-8071 increases p53-DNA binding in BT-474 cells, an effect which is also characteristic of PRIMA-1 [11]. BT-474 cells are known to overexpress mutant p53 [18].

2. Methods

2.1. In silico screening

We used our protein–ligand docking software package MDock [8,9] (zoulab.dalton.missouri.edu/software.htm) to dock PRIMA-1 into many potential drug targets. Although MDock is sufficiently computationally efficient for PDB-wide database screening, we chose to start with the well-characterized Potential Drug Target Database (PDTD), which at the time of use contained about 1100 experimentally determined structures of 830 actual or suspected drug targets (http://www.dddc.ac.cn/pdtd) [19]. We also used the PDTD's binding site definitions, which in most cases are based on the set of amino acid residues that are within 6.5 Å of the bound ligand. OMEGA Version 2.2.1 was used to generate conformations of PRIMA-1 for flexible-ligand docking (OpenEye Scientific Software Inc., Santa Fe, NM) with the *rms* parameter set to 0.1 Å, *maxconfs* to 1,000,000, *maxconfgen* to10,000,000, and *ewindow* to 10. As PRIMA-1 has few rotatable bonds, this only resulted in 42 generated ligand conformations. Each of these conformations was docked to each protein as a rigid body.

Our docking procedure is described in detail in previous publications [8,9,20–22] and in the tutorial of MDock. Briefly, for each protein in the database, a molecular surface of the binding site was generated, along with the associated sphere points representing potential initial positions for ligand atom centers [23,24]. Ligand atoms were matched to these sphere points and orientations were sampled and ranked by our knowledge-based scoring function, ITScore [8,9]. All of MDock's default parameters were used in this work, with the exception of *write_score_total*, which was set to 1 so that only the highest-scoring orientation is recorded when each protein/PRIMA-1-conformation pair is docked as a rigid body. We then ranked each protein according to the lowest ITScore (corresponding to the highest predicted affinity) recorded for any of the 42 PRIMA-1 conformations that were docked to it. Because PDTD contains redundant experimental structures of the same protein [19], we clustered the resulting docked structures into groups sharing ≥90% sequence identity. We then ran a BLAST search [25] in order to map the PDTD proteins, which come from various species, to human gene sequences. Inhibitors of the top human or human analogue proteins were considered candidate anti-cancer agents for assay. A flowchart of our procedure is shown as Fig. 2.

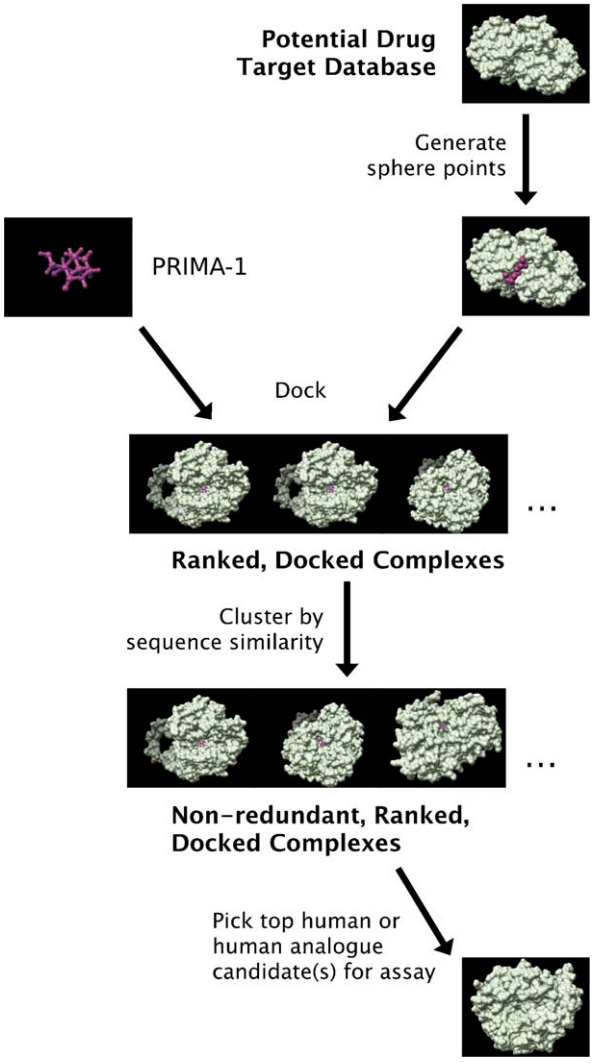

Fig. 2. A flowchart illustrating the inverse docking and assay approach used in this work.

2.2. Cell viability assay

We used the sulforhodamine B (SRB) assay [26–29] to evaluate the effect of the OSC-inhibitor Ro 48-8071 on the viability of breast cancer cells. This cell protein dye-binding assay determines the protein content in surviving cells as an index to determine cell growth,

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