Journal of Molecular Graphics and Modelling 69 (2016) 1–7

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Development and targeting of transcriptional regulatory network controlling *FLU1* activation in *Candida albicans* for novel antifungals

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ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 24 June 2016
Accepted 25 July 2016
Available online 27 July 2016

Keywords:
Candida albicans
FLU1
Regulatory networks
Drug resistance
Antifungals

ABSTRACT

Candidiasis caused by primarily *Candida albicans* poses serious threat due to dry pipeline and ineffective antifungal strategy against resistance. In this study we propose to target genes involved in efflux mediated Multi drug resistance. The main objective of this study was to understand the regulatory interactions responsible for activating a major MFS transporter gene of *Candida albicans*. Another aim was to identify the docking effect of certain antifungal compounds upon the transcription factor effectively controlling *FLU1*. The *in silico* study carried out here aims at control of gene expression at initial levels. This approach helps to understand regulatory control of *FLU1* based on which a predictive map was generated. This data focused on factors with major control that could be suitable target for antifungal agents. The docking results confirm the agreeable effect on the target transcription factor. Broadly this sort of study would account for understanding and targeting any significant gene which in turn would help in adjusting therapeutics accordingly. Further *in silico* ADMET analysis reported positive values that are indicative of a good antifungal compound with respect to pharmacokinetics. These tests are essential in assessment of good drug candidates because they not only help in refining better drug candidates but weeding out the unsuitable ones too.

1. Introduction

Infections caused by fungal pathogens have imposed a serious threat in the last few decades. *Candida albicans* is by far the predominant cause of life-threatening invasive mycoses followed by other *Candida* species (*e.g. Candida glabrata, Candida parapsilosis, Candida tropicalis*) [1,2]. *Candida albicans* is an asexual pathogenic fungus that causes opportunistic infections commonly seen in immunocompromised and debilitated patients [3]. The treatment of fungal diseases relies primarily on the availability of antifungal agents broadly classified as azoles, polyenes, echinocandins, allylamines and pyrimidines [4]. Unfortunately, some of these antifungal drugs have been extensively used and led to an increased selective pressure and the development of antifungal resistance [5]. The major mechanism responsible for high-level azole resistance in clinical *Candida* isolates is overexpression of plasma membrane efflux pumps. There are two main families of efflux proteins, the ATP-binding cassette (ABC) pumps and the major facilitator superfamily (MFS) transporters. *ABC* transporter genes are *CDR1* (*Candida* drug resistance1) and *CDR2* (*Candida* drug resistance 2) which have broader specificity whereas *MFS* transporters comprise large superfamilies of proteins with high sequence similarity found in plants, animals, bacteria, and fungi [6]. Two major genes controlling transport and involved in drug resistance include *CaMDR1* (*Candida albicans* multidrug resistance) and *FLU1* (Fluconazole resistance 1) [7]. Previous studies have reported *FLU1* as major gene involved in resistance, and its disruption makes *Candida* susceptible for antifungal drugs [8].

Due to dearth of antifungal drugs there is an ardent need of new fungal targets. Considering the various potential targets in *Candida*, elements of gene expression hold a rather significant place. Not only the discovery of novel regulatory elements is important but a clear understanding of already documented ones is also crucial. This study will specifically focus on understanding and targeting the transcription factors and their regulatory involvement in activation of *FLU1*.

The estimated size of *Candida* genome is approximately 16MB that comprises of 6354 genes spread over 8 chromosomes; chromosome 1–7 and R. Taking into account the most crucial species *i.e. Candida albicans*, the total coding genes and GC content are 61.5% and 33.7% respectively. A total of 1218 (19.2%) genes encode unique proteins with no significant homologs in the sequence databases[9]. This data gives a clear indication of a number of genes

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http://dx.doi.org/10.1016/j.jmgm.2016.07.009
1093-3263/

being homologues to closely related organisms. *C. albicans* genes have been identified frequently through sequence homology with modal fungal organism *Saccharomyces cerevisiae*. Gene expression is primarily controlled by specific regulatory sequence elements that are recognized by transcription factors (TFs) [10]. In eukaryotes the control of transcription is combinatorial. Several transcription factors bind to the promoter of a particular gene and their complex interplay leads to expression of that gene. Therefore identifying this cooperative interplay is a major aid in drug discovery[11]. In this study we take an account of *FLU1* with respect to its homolog in *Saccharomyces cerevisiae TPO1*.

2. Methods

2.1. Alignment of genome sequence

Genome sequences of *Saccharomyces cerevisiae* and *Candida albicans* (SC5314) are extracted from NCBI or GenBank database. A complete whole genome sequence alignment for both the organisms was carried out using N- BLAST (nucleotide – Basis local alignment search tool) which is a heuristic version of local Smith-Waterman alignment. The Fungal BLAST tool in SGD (*Saccharomyces* genome database) was used where sequences can be submitted for BLAST search in two different ways. It can be uploaded as a file with FASTA or GCG format, or pasted into the query sequence window.

2.2. Alignment of gene of interest

A retrieval of nucleotide sequences for TPO1 from SGD (http://www.yeastgenome.org/) was carried. Similarly complete gene sequence of *FLU1* from *Candida* genome database was retrieved (http://www.candidagenome.org/). Using N-BLAST and fungal BLAST nucleotide sequences of *TPO1* and *FLU1* were aligned and compared for similarity. Alignment could be refined by changing following parameters; protein comparison matrix, expect threshold, word length.

2.3. Transcription factor network building of TPO1

Transcription factor databases such as Yeast Transcription Factor Specificity Compendium-YETFASCO (http://yetfasco.ccbr.utoronto.ca/) and SCPD (http://cgsigma.cshl.org/jian/) were used to get information on all the transcription factors regulating *TPO1*.Yeastract (http://www.yeastract.com/) was used to generate the list of transcription factors involved in activation of desired gene of interest *TPO1* from *Saccharomyces cerevisiae* [12]. A file containing all the best hits of data is available for download.

2.4. Network construction of FLU1

An extensive use of STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database was carried out. Using hits from Yeastract generated file of regulatory proteins, plausible known and predicted protein interactions were projected. In the network view, the database provides an "interactive" mode of the network that has many interesting features, such as automatic layout and clustering by using two different algorithms (KMEANS and MCL) to cluster the proteins that are displaying in the network. Using Fungal Transcription Factor Database (FTFD) an element of CFGB (Comparative Fungal Genomics Platform) (http://cfgp.riceblast.snu.ac.kr) a comparison was made for *Saccharomyces cerevisiae* TF to *Candida* TF. It established similarity in terms of sequence, and overrepresented regulatory sites. The links of all the tools employed are given in methods discussed above. A flowchart for better understanding of the results is provided in Fig. 1.

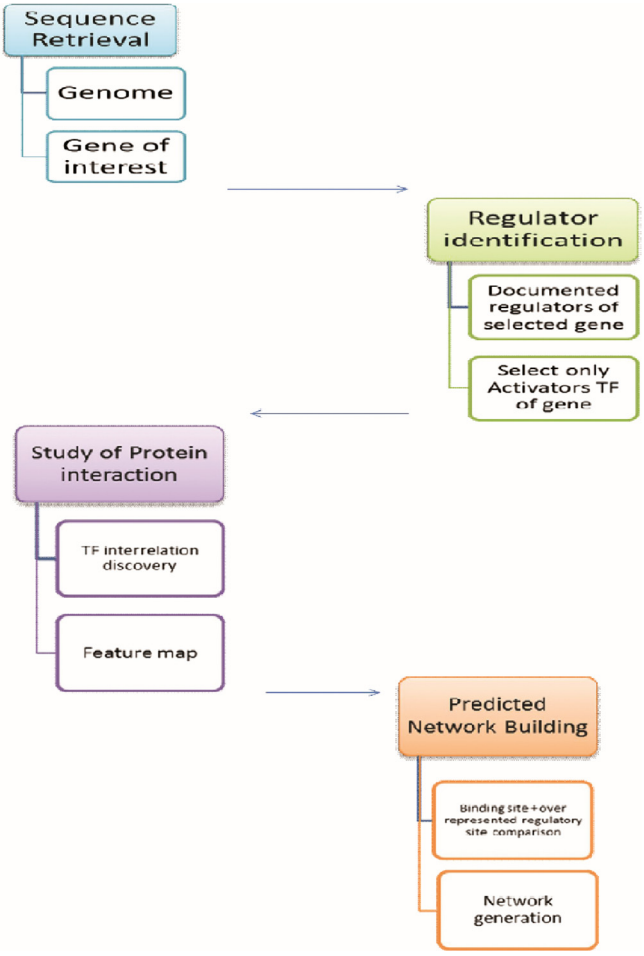

Fig. 1. A layout of framework of stepwise methodology carried out here is arranged in a nutshell.

2.5. Docking of selected transcription factor of FLU1 with antifungals

Of all the available softwares and servers for docking, the freely available and one of the most competent software "AutoDock" was used. AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. It is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Autodock4.2 requires few more softwares to run efficiently and systematically.

CHEMSKETCH along with MDL tools was employed to design the 3D structures of receptor protein as well as ligand antifungal agents. An online server (https://www.molecularnetworks.com/online_demos/corina) was also employed for creating 3D structures of complex chemical compounds. The output file thus generated was in MDL format. It was converted in PDB format using Open BABLE. Docking was performed using Autodock 4.2. For visualization of result PYTHON viewer was employed. Another web server called PatchDOCK was used to confirm and validate results (http://bioinfo3d.cs.tau.ac.il/PatchDock/) [13,14].

2.6. Validation of docking

Acridone derivative compound were further docked against *Candida albicans TPO2*. *C. albicans FLU1* and *TPO2* were found to be the closest homologs of *Saccharomyces cerevisiae TPO1*.