Journal of Computational Science 10 (2015) 186–194

Contents lists available at ScienceDirect

Journal of Computational Science

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

CAVS—Novel in silico selection strategy of specific STAT inhibitory compounds

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ARTICLE INFO

Article history:
Received 31 October 2014
Received in revised form 25 February 2015
Accepted 1 March 2015
Available online 3 March 2015

Keywords:
STAT
Inhibition strategy
Comparative virtual screening
Protein-ligand interactions
Drug identification

ABSTRACT

Signal transducers and activators of transcription (STATs) are a family of proteins activated by different stimulating factors, including interferons, interleukins, growth factors and oncoproteins. Their role in many diseases has been proven, so there is a strong demand to find specific strategies for STAT inhibition. Our searches for specific STAT-targeting compounds focused on exploring the phosphotyrosine (pTyr) SH2 interaction area. Current selection strategies are insufficient, thus, we developed a new pipeline strategy. In this work we used our recently built 3D models for all human (h)STATs (1–4, 5A, 5B and 6). To select specific inhibitors for the STAT protein of interest, we designed and implemented a five step comparative virtual screening tool, which we named – CAVS (comparative approach for virtual screening). CAVS introduces the 'comparative binding affinity value' (CBAV) and 'ligand binding pose variation' (LBPV) as selection criteria to identify specific inhibitors of STATs. In a five-step approach, including pre-screen, primary filtering of inhibitors, re-screen, secondary filtering of inhibitors and graphical inspection and final selection, CAVS leads to selection of specific STAT inhibitory compounds. CAVS was tested on a small ligand library of 130 000 natural products and 5.7 million of clean leads for the selection of STAT1 or STAT3-specific inhibitors. With the use of a designed set of Python scripts for data managing and filtering, CAVS allows to convert the comparative virtual screening procedure into an automatic pipeline and to effectively analyze virtual screening results from standard Surflex-Dock 2.6 output files. We also adapted CAVS as a general-purpose pipeline, which will allow adapting written code to different experiments and protein families in the future.

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1. Introduction

Signal transducers and activators of transcription (STATs) are 79–113 kDa proteins, whose activity is dependent on different stimulating factors, including interferons (IFNs), interleukins (ILs), growth factors like EGF and PDGF and oncoproteins like ABL and Src. In humans seven members have been identified in this family, including STAT1–STAT4, STAT5A, STAT5B and STAT6. Structurally they share five domains: an amino-terminal domain, a coiled-coil domain, a DNA-binding domain, an SH2 domain and a carboxyl-terminal transactivation domain (Fig. 1A) [1]. STATs are involved in the JAK/STAT signaling pathway, controlling programming gene expression in biological events as diverse as: embryonic development, programmed cell death, organogenesis, innate immunity, adaptive immunity and cell growth regulation [2,3].

In general STAT activation is promoted by ligand binding to its receptor, which induces receptor phosphorylation and recruits the STAT proteins to the phosphorylated sites of the receptor. Phosphorylation of the critical tyrosine residue (Tyr) in the STAT protein is then initiated by tyrosine kinases (growth factor receptors, Janus kinases – JAKs and SRC family kinases). Two phosphorylated STAT monomers dimerize through reciprocal pTyr-SH2 domain interactions (Fig. 1B), and the STAT homodimers translocate to the nucleus where they bind to specific STAT-response elements in the target gene promoters and regulate transcription [3].

For more than ten years STATs are of particular interest to the scientists. Accumulating evidence strongly implicates their role in diseases connected to immune system malfunction, e.g., infection and immune disorders (STAT1 and STAT2), cancer (STAT2, STAT3, STAT5A and STAT5B), cardiovascular diseases (STAT1), asthma and

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http://dx.doi.org/10.1016/j.jocs.2015.03.001
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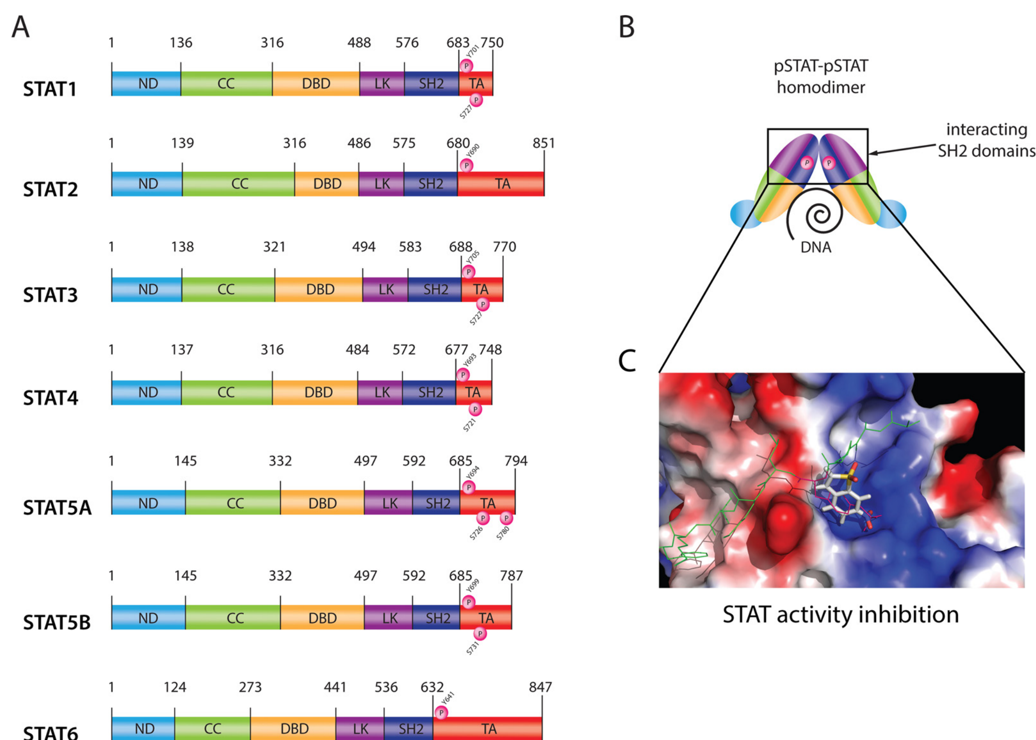

Fig. 1. A – Functional domains of human STAT proteins. ND: N-terminal domain; CC: coiled-coil domain; DBD: DNA-binding domain; LK: linker domain; SH2: src-homology 2 domain; Y-P: phosphorylated tyrosine; S-P: phosphorylated serine; TA: transcriptional activation domain. B – Homodimer of phosphorylated STAT binding to DNA. Colors of domains are according to those assigned under A. Dimerization involves interaction between the phosphorylated tyrosine of one STAT1 molecule and the SH2 domain of the dimer partner in a parallel orientation that is a prerequisite for DNA binding. C – STAT activity inhibition example. SH2 domain-based competitive small inhibitor for hSTAT3, stattic, docked to the phosphotyrosine-(pTyr)-binding pocket in hSTAT3-SH2 domain. Stattic is shown in stick representation, pTyr-linker is presented as lines colored in green with pTyr residue colored in pink. Results were obtained using Surflex-Dock 2.6 program. hSTAT3-SH2 domain is in the surface representation, colored according to the distribution of the electrostatic surface potential, calculated with APBS. Blue indicates positively charged regions, red indicates negatively charged regions.

allergy (STAT6), systemic lupus erythematosus (STAT4) and chronic myelogenous leukemia (STAT5A and STAT5B) [4]. STAT inhibitors therefore could be valuable in treatment of these diseases and various STAT inhibitory strategies have been pursued, particularly for STAT3, due to its vast connection with nearly 70% of cancers [5].

2. Related work

Most of STAT-targeting strategies focus on inhibiting STAT3 dimerization using miscellaneous small compounds, biopolymers and macromolecules [4–11]. Searches for STAT3-targeting compounds, exploring the pTyr-SH2 interaction area of STAT3, are especially numerous and yielded many small molecule inhibitors (Fig. 1C) [4–6,8,10]. In contrast, only a few inhibitors for other STATs are described [4]. We are especially interested in finding STAT1 and STAT3 specific inhibitors, because of their opposite roles in inflammation and tumorigenesis [12,13], despite high structural conservation – they share nearly 50% of identical amino acids [14].

Selection strategies of SH2 domain-based competitive small inhibitors for STAT3 and other STATs have been summarized in works of Miklossy et al., Debnath et al., Deng et al., Furqan et al. and Lavecchia et al. [4–6,8,10]. However, identifying STAT-specific inhibitors meets many challenges, like insufficient structural data, undefined mechanism of action and poor in vivo performance of selected compounds. Moreover, many of these inhibitors seem not STAT-specific, thereby questioning the present selection strategies of SH2 domain-based STAT inhibitors. For example, Bill et al. proved the non-specificity of curcumin toward STAT3 and provided evidence of its cross-binding to STAT3 and STAT1 [15]. This also accounted for other STAT3-targeting compounds: BP-1-102 [16], cryptotanshinone [17], Cpd30-12 [18], cyclopentenone derivatives [19], OPB-31121 [20], resveratrol analogs (RSVA314 and RSVA405) [21] and S3I-201 [5]. Growing evidence on STAT cross-binding illustrates the need for better models, and screening and validation tools for more drugable STAT inhibitors with high specificity, potency and excellent bioavailability.

In our studies we concentrated on the problem of STAT cross-binding specificity of some STAT-selective compounds. By generating new models for human STAT1–STAT3 we applied comparative in silico docking to determine SH2-binding specificity of the STAT3 inhibitor stattic, and of fludarabine phosphates (STAT1 inhibitor). We provided evidence that by primarily targeting the highly conserved phosphotyrosine SH2 binding pocket, stattic is not a specific STAT3 inhibitor, but equally effective toward STAT1 and STAT2. This was confirmed in human micro-vascular endothelial cells (HMECs) in vitro, in which stattic inhibited IFNγ-induced phosphorylation of all three STATs. Likewise, we proposed that fludarabine (Flu) phosphates inhibited both STAT1 and STAT3 phosphorylation, but not of STAT2, by competing with the highly conserved binding sites, which are less well-preserved in STAT2. In accordance with this we observed that in HMECs in vitro Flu inhibited cytokine and LPS-induced phosphorylation of STAT1 and STAT3 but had no influence on STAT2 phosphorylation. Our previous data offer a molecular basis that explains STAT cross-binding specificity of stattic and fludarabine phosphates, thereby questioning the present selection strategies of SH2 domain-based competitive small inhibitors [22].

To examine the binding specificity of a pre-selection of STAT3 inhibitors (cucurbitacin E, cucurbitacin Q [23], curcumin [24], stattic [25], LLL12 [26], Cpd188, Cpd30-12 [18], STX-0119 [27], S3I-201 [28], S3I-201.1066 [29], BP-1-102 [30], WP1066 [31], FLLL32 [32], HJC0123 [33] and OPB-31121 [20]) recently we also generated 3D

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