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# Protein–drug interactome analysis of SSRI-mediated neurorecovery following stroke

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ABSTRACT

Serotonin selective reuptake inhibitors (SSRIs) have been widely used as first-line drugs in the treatment of a range of depressive and anxiety disorders. Recently, clinical studies found that this class of agents also shows significant efficacy in promoting neurogenesis, neuroplasticity and neurorecovery following stroke. Here, we attempt to elucidate molecular mechanism and biological implication underlying the SSRI-mediated neurorecovery. In the procedure, a comprehensive protein–drug interactome (PDI) was constructed for various SSRIs and their major metabolites as well as a group of control drugs across a large panel of human neuroproteins *via* a high-throughput molecular docking approach. The obtained PDI was then analyzed at systematic level to extract unexpected targets for SSRIs/metabolites. Biological network analysis and gene ontology (GO) enrichment solidified that the inferred targets have high potential to be directly or indirectly involved in diverse neural events, and further molecular dynamics (MD) simulation and post molecular mechanics-Poisson Boltzmann/surface area (MM-PB/SA) characterization revealed a stable complex architecture and high-affinity interaction between the targets and SSRIs/metabolites. Specifically, two human proteins, i.e. neurogenic locus notch homolog protein 1 (NOTCH 1) and Rho-associated protein kinase 1 (ROCK 1), were suggested as promising regulators in the SSRI-mediated neurorecovery, which can be targeted efficiently by fluoxetine and paroxetine, respectively, as well as other SSRIs and metabolites.



## 1. Introduction

The serotonin selective reuptake inhibitors (SSRIs) are a class of psychotropic drugs clinically used as antidepressants in the treatment of depression, anxiety disorders and other conditions as well (Stahl, 1998). These drugs have established a pathophysiological role of serotonin (5-HT) in affective disorders and the spectrum of anxiety disorders; they are also the first to confirm the inhibition of neurotransmitter reuptake as an important therapeutic strategy. As a result, the discovery of these agents marks a milestone in neuropsychopharmacology and rational drug design (Vaswani et al., 2003).

SSRIs are traditionally used to treat depression and psychiatric disorders. However, it has been observed that this class of drugs can also cause various adverse drug reactions (ADRs), including nausea, headache, insomnia, tremor, mania, diarrhea, paresthesia, weight loss and, in few cases, sexual dysfunction (Demyttenaere and Jaspers, 2008). Obviously, the binding of SSRIs to their cognate targets, e.g. dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT), cannot be all responsible for such a wide spectrum of side effects, and some researchers thus proposed that the ADRs might be elicited by targeting unexpectedly a variety of ADR-mediating proteins that do not belong to any kind of known therapeutic targets (Yang et al., 2009, 2010). On the other side, the SSRIs have long been recognized to exert potential effects on the neuroplasticity and cognitive recovery of patients suffering from stroke (Glodstein, 1998). Malberg et al. (2000) early observed that adult rats with administration of several different classes of antidepressant agents increased the proliferation of hippocampal cells and that these new cells mature and become neurons, indicating that increased neuronal number may be a mechanism by which antidepressant treatment overcomes the stress-induced atrophy and loss of hippocampal neurons. Recently, both clinical studies and epidemiological investigations suggested a positive role of many SSRIs and their derivatives in neurogenesis during stroke recovery. For example, by survey of 129 patients within 3 months following stroke it was found that the patients who received escitalopram showed improved performance in neuropsychological tests assessing memory and executive functions compared to those who received placebo or underwent problem solving

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therapy (Jorge et al., 2010). In addition, substantial improvement was reported for the patients of acute ischemic stroke after treated with fluoxetine as compared to those with placebo, albeit some adverse events such as hyponatraemia and transient digestive disorders were observed in the fluoxetine group (Chollet et al., 2011).

Although there is a significant statistical correlation between the SSRI administration and improved stroke recovery, the underlying molecular mechanism and biological implication still remain largely unexplored. Here, we hypothesize that (i) the pharmacological effect of SSRIs on neurorecovery following stroke by targeting unexpectedly certain functional proteins in the central nervous system; these proteins are commonly the releaser, carrier, regulator and receptor of neurotransmitters as well as enzymes, transcription factors and ion channels, and (ii) due to the complexity of neurogenesis and neuroplasticity this effect may not be achieved effectively by the binding of SSRIs to only one or few proteins, and multiple targets would therefore be expected in the SSRI-mediated neurorecovery. In order to test our hypotheses and to identify the unexpected targets of SSRIs, in the current study we constructed a comprehensive protein–drug interactome (PDI) for various SSRIs and their major metabolites across a large panel of human neuroproteins *via* a high-throughput molecular docking approach. The obtained virtual interactome was then examined systematically to extract two subsets separately of drug and protein candidates involved in the SSRI-mediated neurorecovery. We also analyzed biological interaction networks and enriched gene ontology terms associated with inferred targets, and performed molecular dynamics simulation and binding free energy examination to characterize the structural basis and energetic property of several promising protein–drug interaction pairs. This work would promote our understanding of biological principle underlying the SSRI-mediated neurorecovery following stroke.

## 2. Materials and methods

### 2.1. Compilation of data sets

#### 2.1.1. Structure-based set of pocket-containing human neuroproteins

The term neuroprotein is a comprehensive term covering all proteins that directly or indirectly participate in various nervous processes. Here, we only selected those that are directly involved in neuron behavior and synaptic activity. Currently, a total of 3249 human neuroproteins are recorded in the SynDB database (Zhang et al., 2007), to which we carried out an exhaustive FASTA search (Pearson and Lipman, 1988) against the PDB database (Berman et al., 2000) to extract 876 candidates with solved high-resolution structures. Subsequently, the geometry-based SURFNET algorithm (Laskowski, 1995) was implemented to identify ligand-binding pockets on the surface of these extracted candidates; SURFNET detects the void regions in proteins by fitting spheres into the spaces between protein atoms and the sphere fitting process results in a number of separate groups of interpenetrating spheres, which correspond to the cavities and clefts of the protein (An et al., 2005). We also employed manual approach to exclude those cases that are obviously not suitable for SSRIs to bind. For example, the SSRI molecules contain a number of bulky aromatic rings, and hence we excluded those of narrow pockets. As a result, 472 neuroproteins were consequently found to have 526 potential pockets capable of accommodating small-molecule ligands, and in these pockets 34 co-crystallized compounds were identified. Although this neuroprotein set was incompetent to cover the whole SSRI targets, if some unexpected and valuable information could be mined from it, the PDI would enlighten the following research and lead to the construction of a large-scale target set. The 472 compiled neuroproteins are summarized in Supporting Information Table S1.

Supplementary Table S1 can be found, in the online version, at http://dx.doi.org/10.1016/j.biosystems.2014.03.007.

#### 2.1.2. FDA-approved SSRIs and their major metabolites

Up to date, there are nine SSRIs that have been approved by US FDA, including citalopram, dapoxetine, escitalopram, fluoxetine, fluvoxamine, indalpine, paroxetine, sertraline and zimelidine. Since the citalopram is a racemic mixture and its (*S*)-stereoisomer is escitalopram, here we only investigated the escitalopram but discarded the citalopram. In addition, 10 reported major metabolites of these SSRIs were also included in this set; most of SSRI metabolism *in vivo* are demethylation reactions exerted by cytochrome P450 (CYP2D6) (Hemeryck and Belpaire, 2002). The structures of the 18 SSRIs and metabolites are listed in Table 1.

#### 2.1.3. Control drug set

From the DrugBank database (Wishart et al., 2006) we randomly selected 30 FDA-approved small-molecule agents to define a control set. The selected control drugs stratified: (i) they possess molecular weights and sizes close to the SSRIs, (ii) their cognate targets do not belong to the generalized family of neuroproteins, and (iii) they have never been reported to have pharmacological effects and adverse drug reactions on nervous system. This control drugs and their comparisons with the 18 SSRIs are provided in Supporting Information Table S2.

Supplementary Table S2 can be found, in the online version, at http://dx.doi.org/10.1016/j.biosystems.2014.03.007.

### 2.2. High-throughput molecular docking

First, the co-crystallized water molecules and cofactors were removed from the crystal structures of the 472 neuroproteins and the polar hydrogen atoms were added to protein structures. Then, the atoms of protein receptors and ligand molecules were assigned with Kollman (Singh and Kollman, 1984) and Gasteiger (Gasteiger and Marsili, 1980) partial charges, respectively. In docking procedure, the AutoDock Tools (Morris et al., 2009) were employed to set the center and size of grid boxes covering the identified ligand-binding pockets on protein surfaces, and to prepare the *pdbqt* files for proteins and ligands. Here, the center and size of grid boxes should completely cover the whole ligand-binding pockets on protein surfaces. In practice, the boxes were slightly larger than the pockets in each of *x*, *y* and *z* dimensions so that the later docking performance can fully search conformational space for ligand molecules within the protein pockets. The docking calculations were implemented with AutoDock Vina (Trott and Olson, 2010), which utilized Lamarckian genetic algorithm (LGA) to explore the conformational space of ligand molecules within the active pocket of protein receptors. In order to facilitate the large scale docking of 82 ligand molecules (including 34 co-crystallized compounds, 18 SSRIs/metabolites and 30 control drugs) into 526 protein pockets in the neuroprotein set (Fig. 1), the GUI interface AUDocker LE (Sandeep et al., 2011) was applied to automating and speeding up this process.

### 2.3. Atomistic molecular dynamics simulation

The docked complex structures and their binding free energies of few promising neuroprotein–SSRI interaction pairs were characterized by molecular dynamics (MD) simulations using AMBER03 force field implemented in the AMBER9 program package (Case et al., 2005). The complex systems were first relaxed with 10,000 cycles of molecular mechanics (MM) minimization; the first 2000 steps were performed with the steepest descent algorithm, whereas the rest of the steps were performed with the conjugate gradient algorithm. Subsequently, MD simulations with a target