Supplementary Material

Targeting AMPK Signaling Pathway with Natural Medicines for Atherosclerosis Therapy: An Integration of In Silico Screening and In Vitro Assay

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Abstract: The activation of AMP-activated protein kinase (AMPK) signaling pathway plays a central role in the pathologic progression of atherosclerosis (AS). Targeting the AMPK is thus considered as a potential therapeutics to attenuate AS. Here, we report the establishment of a synthetic pipeline that integrates *in silico* virtual screening and *in vitro* kinase assay to discover new lead compounds of AMPK inhibitors. The screening is performed against a large-size pool of structurally diverse natural products, from which a number of compounds are inferred as promising candidates, and few of them are further tested *in vitro* by using a standard kinase assay protocol to determine their inhibitory potency against AMPK. With this scheme we successfully identify five potent AMPK inhibitors with IC₅₀ values at micromolar level. We also examine the structural basis and molecular mechanism of nonbonded interaction network across the modeled complex interface of AMPK kinase domain with a newly identified natural medicine.

Complex Structures

Figure S1. The complex structure architectures of AMPK kinase domain with five high-activity natural products: baicalin (A), balanol (B), gossypol (C), emodin (D) and cercosporamide (E). These complex structures were modeled using molecular docking calculations and then equilibrated with MD simulations.

Materials and Methods

Natural product library

More than 100,000 natural products were extracted from in the ZINC (Irwin & Shoichet 2005), UNPD (Gu et al. 2013) and CamMedNP (Ntie-Kang et al. 2013) databases. The molecular structures of natural products were characterized by using daylight fingerprints, and a heuristic clustering strategy was used to analyze structural diversity among these compounds. In this way, those with high structural similarity were excluded from the initial natural product pool. The PreADMET method (Lee et al. 2004) was then employed to evaluate drug likeness for the pool, and ultimately 24,709 compounds passed the evaluation and they constituted a distinct library of natural products with diverse structure and high drug-likeness, which will be used in subsequent high-throughput virtual screening of potent AMPK inhibitors. Here, the three-dimensional structures of these compounds were constructed using CORINA program (Sadowski et al. 1994) and then minimized by MMFF94 force field (Halgren 1996).

Molecular docking

DOCK. The rigid docking (RGD) protocol (Mukherjee et al. 2010) was employed here to perform molecular docking of the 24,709 natural product compounds into the active site of AMPK kinase domain by using UCSF DOCK method (Meng et al. 1992). The protocol was modified from a previous work (Powers et al. 2002). In the protocol, the high-resolution crystal structure of AMPK kinase domain bound with its cognate inhibitor compound C (PDB: 3AQV) was used as template to perform molecular docking. To prepare the site for docking, all water molecules and ions as well as the cocrystallized compound C ligand were removed from the crystal structure system. The sphere set was generated based on ligand atom positions from an ensemble of structure of the compound C in complex with AMPK, resulting in a cluster of 87 spheres, and each of them represented a ligand atom position observed in the crystal structure used in the docking calculation. Calculated interaction energies in

the docking calculations were corrected for ligand desolvation (Shoichet et al. 1999), which was also used to calculate ligand partial atomic charges. Each orientation of the docked ligands was refined with 100 iterations of rigid-body minimization (Meng et al. 1993).

Autodock. The flexible Autodock method (Goodsell & Olson 1990) was further used to analyze and screen those promising AMPK binders suggested by DOCK. The binding site for the calculations was defined using the cocrystallized compound C in AMPK kinase domain. Gasteiger and Kollman united atom charges were assigned for natural product ligands and AMPK protein, respectively. The protein was also added with polar hydrogen atom. The affinity grids centered on and encompassing the active site were calculated for the relative ligand atom types with a 0.375 Å the spaced box size was set $x = 70$, $y = 55$ and $z = 50$ Å. The torsion and rotatable bonds in the ligands were defined, and the nonpolar hydrogens and partial atomic charges were also added to the bonded carbon atoms. A flexible docking was carried out with AutoDock Vina (Trott & Olson 2010) to evaluate ligand binding energies over the conformational search space using the Lamarckian genetic algorithm (Ni et al. 2011).

Molecular dynamics simulations and binding free energy analysis

Molecular dynamics (MD) simulations were carried out using AMBER FF03 force field (Duan et al. 2003) in AMBER11 package (Case et al. 2005). The force field parameters and partial charges of ligand molecules were assigned by using generalized AMBER force field (GAFF) (Wang et al. 2004) and restricted electrostatic-potential fitting (RESP) approach (Bayly et al. 1993), respectively. A truncated octahedral box of TIP3P waters (Jorgensen et al. 1983) was added with a 10 Å buffer around the complex. The counter-ions were added to maintain the electroneutrality of both simulated system. Complex systems were heated to 300 K in 100 ps followed by constant temperature equilibration at 300 K for 300 ps. Subsequently, 10-ns MD production simulations were carried out for both systems in an isothermal isobaric ensemble with periodic boundary conditions. An integration step of 2 fs was used for the MD simulations and the particle mesh Ewald method (Darden et al. 1993) was employed to calculate the long-range electrostatic

interactions. A cut-off distance of 10 Å was used to calculate the short-range electrostatics and van der Waals interactions. In order to restrain all covalent bonds involving hydrogen atoms, the SHAKE method (Ryckaert et al. 1977) was used. Each simulation was coupled to a 300 K thermal bath at 1.0 atm through the Langevin algorithm (Wu & Brooks 2003).

The snapshots extracted from production simulation were used to perform free energy analysis for ligand binding to AMPK kinase domain, which was carried out by molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) (Kollman et al. 2000). This method considered the total binding free energy (ΔG_{total}) made up of direct nonbonded interaction (ΔE_{int}) between the protein and ligand and indirect solvent effect (ΔG_{solv}) due to the removal of water molecules from the protein–ligand complex interface.

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