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Original article

Ligand selectivity of estrogen receptors by a molecular dynamics study

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ABSTRACT

Estrogen receptors α (ERα) and β (ERβ) have different physiological functions and expression levels in different tissues. ERα and ERβ are highly homologous and have only two residue substitutions in the binding pocket. This high similarity at the active site stimulates the interests for discovering subtype selective ligands. In this study, molecular dynamics (MD) simulations combined with molecular mechanics generalized Born surface area (MM-GBSA) method have been carried out to analyze the basis of selectivity of three ligands (**659**, **818** and **041**). The calculated binding free energies show that all the ligands bind more tightly to ERβ than to ERα. The dominant free energy components of selectivity for **659** are similar to that for **041**, but different from that for **818**. The decompositions of free energy contributions and structural analysis imply that there are eight residues primarily contributing to the selectivity for **659**, five residues for **041**, as well as two residues for **818**. The structural analysis implies that two residue substitutions in binding packet cause the position of **659** in ERβ–**659** complex to shift relative to that in ERα–**659** complex and also cause the conformational changes of other residues in the binding pocket. The higher selectivity for **041** is mainly caused by three residues, Ile373 (Met421), His475 (His524) and Leu476 (Leu525).

1. Introduction

Estrogen receptors (ERs), which are members of the nuclear receptor (NR) superfamily of ligand regulated transcription factors, mediate the physiological effects of both endogenous and synthetic estrogens [1,2], and their targets include reproductive tissues, the brain, bone and the cardiovascular system [3–5]. To date, there are two forms of estrogens, ERα and ERβ encoded by different genes [6,7]. ERα is well characterized as a mediator of cell proliferation, especially in breast cancer cells, driving proliferation in the presence of estrogen [8]. In opposite to ERα, ERβ inhibits ERα mediated proliferation in many cells [9–12]. The expression pattern of the two ER subtypes is different: ERβ is predominantly expressed in the ovary, prostate, and other tissues including the central nervous system, cardiovascular system, gastrointestinal system, and immune system [13,14], while ERα is predominantly expressed in breast, thymus, uterus, liver and vagina pituitary [2,15]. The different tissue distributions of the two ER subtypes and their different effectiveness as transcription regulators suggest that each subtype will have different functions [13,16,17]. Therefore, ERs are regarded as important drug targets for many diseases.

Ligands with selectivity for ERβ are now recognized as a new and promising strategy for cancer treatments with the antiproliferative role of ERβ in many tissues. ERβ selective ligands hold therapeutic promise in colon and breast cancers, as well as ovarian and prostate cancers. Selective inhibitors for ERβ could promote ERβ mediated growth inhibition while avoiding proliferative side affects mediated by ERα. However, the design of highly ERβ selective ligands has proved to be quite challenging [9]. ERα and ERβ are highly homologous and share 56% sequence identity in their ligand binding domain (LBD) [18]. Crystallographic structures of the LBD of ERα and ERβ reveal that ERα and ERβ share a high degree of similarity in residues that line the binding cavity, which makes the design of highly potential and selective ligands difficult. Actually, there are only two residues differences in the binding cavity: Leu384 of ERα replaced by Met336 of ERβ and Met421 of ERα replaced by Ile373 of ERβ (Fig. 1a). The differences in expression levels and function between two subtypes stimulate the interests for discovering subtype selective ligands.

To date, a large number of selective ligands utilizing various scaffolds have been reported, such as tetrahydrochrysenes [19], diarylpropionitriles [20], biphenyls [21], benzopyran [22], indazoles [23] and chromenoquinoline [24]. Some of the inhibitors have

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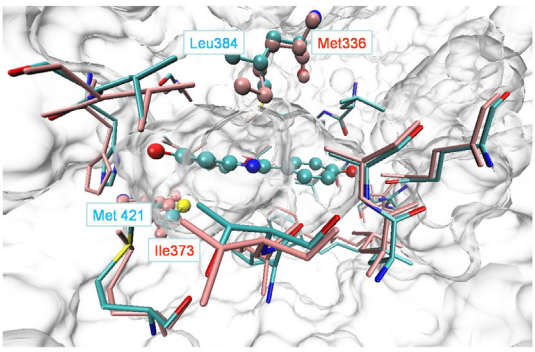

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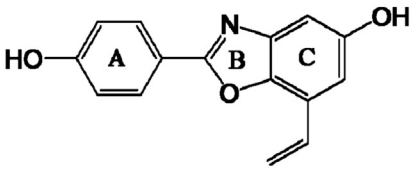

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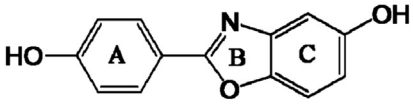

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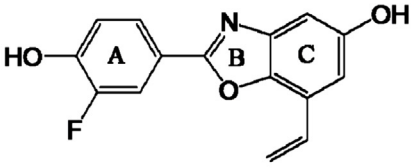

(d)

Fig. 1. (a) Key residues of ERα overlapped with ERβ in the binding pocket. Ligands and various residues in ERα (colored with name) or ERβ (colored pink) are shown in stick and ball representation; molecular structures of ligands **659** (b), **818** (c) and **041** (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

been successful at enhancing ERβ selectivity beyond 50-fold. The availability of highly selective ligands has enabled us to identify the physiological function of ERβ. Selective ERβ agonist ERβ might exert its function in intestinal and joint inflammation and inhibit breast cancer cell proliferation [25–27]. Many previous works tried to interpret the possible mechanism of ligand selectivity in ERs isoforms. Some works focused on the various two residues in the binding pocket have suggested that ERα selectivity was mainly driven by ERα Met421 [28,29], whereas ERβ selectivity could be ascribed to Met336 [30,31]. The studies on ERs chimeras generated by DNA shuffling method have implied that the selectivity for diarylpropionitrile was mainly driven by several residues in helix 3 of the ERβ [20]. The selectivity of ligand propylpyrazole triol was partially driven by residues from helix 8 to the middle part of helix H11, and the long-range interactions played important roles in determining the ligand selectivity [32]. A study by using comparative molecular field analysis has revealed that the electrostatic field played a more important role in ERβ than that in ERα [28]. A report with pharmacophore models has shown that the hydrophobic and hydrogen bonding interactions were essential for the subtype selectivity [33]. Manas et al. investigated a series of ERβ selective ligands, and determined the crystallographic structures [30]. Yun Tang et al. [34] investigated the mechanism of selectivity of **244** (described in Manas's work) based on molecular dynamics (MD) simulations. Their work showed that His524/475 in ERα/ERβ acted as a "gatekeeper" during the ligand unbinding [35] by using steer molecular dynamics simulation. A recent study by using quantitative structure–activity relationship (QSAR) and docking methods has delineated that the size and shape descriptors are best modulators of ERβ selectivity [36].

As described in Manas's work, the ligand selectivity of more than 100 folds are mainly caused by different electronic interaction between ligand and ERα Met421 side chain relative to the side chain of ERβ Ile373. The ligands **659** (Fig. 1b), **818** (Fig. 1c) and **041** (Fig. 1d) have 127.3-, 24.2- and 255.5-folds selectivity for ERβ relative to ERα, respectively, while the **818** molecule differs from **659** only in the absence of a vinyl group. Hence, **818** and **659** ligands were selected to evaluate the ligand selectivity of ERβ relative to ERα and the function of vinyl group, and **041** ligand was also evaluated for the highest selectivity in the series of ligands [30].

MD simulation could serve as a powerful tool for understanding ERβ selectivity over ERα on ligands [34,35,37]. Several computational methods with various levels of computational expense and accuracy exist to estimate ligand binding affinities and selectivities, such as, thermodynamic integration (TI), the free energy perturbation (FEP) method [38], and molecular mechanics generalized Born surface area (MM-GBSA) method [39]. Although the TI and FEP methods should give more accurate binding free energies, they require sufficient statistical samplings and are extremely time-consuming. The heavy computational cost prevents FEP and TI from being routinely used for free energy calculations in structure-based drug design [40]. MM-GBSA/PBSA methods are a versatile tool for calculating the binding free energies of a given protein–ligand complex, which incorporates the effects of thermal averaging with a force field/continuum solvent models to post-process a series of representative snapshots from MD trajectories [41–45].

In this study, MD simulations followed by molecular mechanics generalized Born surface area (MM-GBSA) [39] analysis have been performed to clarify the selectivity of three ligands (**659**, **818** and **041**) (Fig. 1b–d) binding to ERα and ERβ. Both ligands (**659** and **041**) exhibit highly selectivity of ERβ versus ERα [30]. Free energy decomposition method has been used to calculate the detailed binding free energies between these ligands and individual protein residues [39]. The analysis of detailed interaction energies provides insight on the protein inhibitor binding mechanism and helps elucidate the basis for achieving selectivity through interpretation of the structural and energetic results from the simulation. We expect that this study will be helpful for the rational design of potential and selective ligands of these two isoforms.

2. Results and discussion

2.1. Equilibrium of the dynamics simulation

We examined the initial structures obtained by using replacement method and docking method. The ligand **659** was docked into the binding pocket of ERα and ERβ using Dock 6.5 software [46,47]. As seen in Fig. 2, the replaced structure was in agreement with the docked structure. In the following MD simulations, all the complex structures obtained by replacement were used as initial structures.

To assess the dynamics stability of all the complexes during the MD simulations, energetic and structural properties were monitored during the entire MD simulation of each complex. Root-