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Inhibition of Cancer Cell Growth by Baicalein via ESR1 Based on Molecular Dynamics Simulations

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Abstract: This study investigates the mechanism by which baicalin inhibits cancer cell growth through estrogen receptor 1 (ESR1) using molecular dynamics simulations. The results show that baicalin primarily binds to the ligand-binding domain (LBD) of ESR1, interacting through hydrogen bonds and hydrophobic interactions. After binding, the overall and local conformations of ESR1 change, affecting its interactions with other proteins and thus modulating the signaling pathways of cancer cells. Binding free energy analysis indicates that the binding of baicalin to ESR1 is spontaneous and relatively stable. Additionally, baicalin can inhibit the binding of ESR1 to estrogen, blocking the estrogen signaling pathway and thereby suppressing the growth and proliferation of cancer cells. This study provides theoretical and experimental foundations for the potential use of baicalin as an anticancer drug, offering new insights and methods for the development of novel anticancer drugs. However, the study has some limitations, such as limited simulation time and simplified systems. Future research can extend the simulation time and consider more physiological factors to more accurately simulate the interactions between baicalin and ESR1.

Keywords: Baicalin; ESR1; Molecular dynamics simulation; Cancer cell growth; Estrogen signaling pathway

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1. Introduction

Cancer, a major threat to human health globally, remains a focal point in medical research [1]. Traditional cancer treatments, such as surgery, radiation therapy [2], and chemotherapy [3], while effective to some extent, often come with severe side effects. Therefore, the search for efficient and low-toxicity novel anticancer drugs has become a hot topic in current cancer research. Baicalin, a flavonoid compound widely found in *Scutellaria baicalensis* and other traditional Chinese medicines, exhibits various biological activities [4], including antioxidant [4], anti-inflammatory [5], and antibacterial properties [6]. Recent studies have suggested that baicalin also has potential anticancer effects, although the specific mechanisms remain unclear.

Estrogen receptor 1 (ESR1) plays a crucial role in the development and progression of various cancers [7]. Research has shown that ESR1 is overexpressed in breast cancer, endometrial cancer, and other types of cancer, and is closely associated with the growth, proliferation, and metastasis of cancer cells [8]. Cancer cells with ESR1 mutations are relatively resistant to endocrine therapy and are sensitive to ESR1 depletion. Although ESR1 mutations were not prominent in the initial cancer genome atlas due to sequencing of primary untreated tumors, they are evident in 4% of untreated endometrial cancer cases. This highlights the importance of sequencing metastatic and resistant tumors to identify molecular mediators of disease progression. The prevalence of ESR1 mutations in patients depends on the duration and context of previous endocrine therapy. For instance, about 20–40% of patients with metastatic breast cancer (MBC) treated with aromatase inhibitors (AIs) have ESR1 mutations, with varying rates depending on the site of metastatic disease. Therefore, inhibiting the activity of ESR1 has emerged as a promising strategy for cancer treatment.

Molecular dynamics simulation is a computational method based on Newtonian mechanics that simulates the motion and interactions of molecules at the atomic level [9]. Molecular docking and molecular dynamics simulations (MDS) are collectively referred to as molecular simulations. According to the literature, molecular simulation methods, primarily based on computer platforms, can visually identify molecular structures. These methods can accurately predict the interaction modes between different molecules at the atomic level, which is difficult to achieve *in vitro* experiments [10]. Molecular simulations have been proven to save substantial experimental materials and reduce the time and effort required for repetitive experiments. They have been applied in the medical field to screen active small molecules. Through molecular dynamics simulations, one can gain deep insights into the binding mechanisms, conformational changes, and biological impacts of drugs on receptors [11]. This study utilizes molecular dynamics simulation methods to investigate the mechanism by which baicalin inhibits cancer cell growth through ESR1.

2. Materials and methods

2.1. Molecular dynamics simulation software

This study uses Yasara software for molecular dynamics simulations. Yasara is a widely used software in biophysical simulations, known for its powerful force fields and efficient algorithms, enabling accurate simulation of the structure and dynamics of biomolecules.

2.2. Preparation of receptor and ligand structures

2.2.1. Acquisition of ESR1 receptor structure

The crystal structure of ESR1 (PDB ID: 1sj0) was downloaded from the Protein Data Bank (PDB). This high-resolution crystal structure provides an accurate initial structure for molecular dynamics simulations.

2.2.2. Construction of baicalin ligand structure

The two-dimensional structure of baicalin was constructed using ChemDraw software, followed by quantum chemistry calculations using Gaussian09 to optimize its structure. The optimized baicalin structure was then imported into Amber18 software for parameterization.

2.3. Molecular dynamics simulation process

2.3.1. System construction

The ESR1 receptor and baicalin ligand were placed in a cubic box with periodic boundaries. The box size was determined based on the size of the receptor and ligand and the precision requirements of the simulation. Water molecules were added to the box to simulate the physiological environment.

2.3.2. Force field selection

The AMBER14 force field was used to describe the atomic interactions between the protein and the ligand. This force field has been extensively validated and can accurately simulate the structure and dynamics of biomolecules.

2.3.3. Energy minimization

The constructed system was subjected to energy minimization to eliminate unreasonable conformations and atomic contacts. Energy minimization was performed using a combination of steepest descent and conjugate gradient methods until the system's energy reached a convergence criterion.

2.3.4. Heating process

The energy-minimized system was gradually heated from 0 K to 298 K (physiological temperature). The heating process was carried out in a stepwise manner, with equilibrium simulations performed at each temperature increment to ensure system stability.

2.3.5. Equilibrium simulations

Long-term equilibrium simulations were conducted at 298 K to ensure the system reached a stable state. During equilibrium simulations, pressure and temperature were controlled to maintain physiological conditions.

2.3.6. Production simulations

Production simulations were performed based on the equilibrium simulations. The simulation time was determined as needed. The time step for the simulation system was set to 2.0 fs. Trajectory files were saved every 100 ps. The simulation was extended for 100 ns following constant pressure and the Berendsen thermostat.

2.4. Data analysis methods

2.4.1. Binding free energy calculation

The binding free energy of baicalin to ESR1 was calculated using the molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) method. Binding free energy is a critical indicator of the strength of drug-receptor binding, with lower values indicating more stable binding.

2.4.2. Conformational analysis

Conformational analysis was performed on the trajectory files obtained from production simulations, including changes in the conformation of the receptor and ligand, hydrogen bond formation, and hydrophobic interactions. Conformational analysis can reveal how the drug interacts with and affects the receptor.

3. Results and discussion

3.1. The binding mode of baicalein and ESR1

3.1.1. Combining site analysis

As shown in **Figure 1**, molecular dynamics simulation found that baicalein mainly binds to the ligand binding domain (LBD) of ESR1. The specific binding sites include amino acid residues LEU346, GLU353, ARG394, LEU387, etc. These amino acid residues play important roles in the structure and function of ESR1, and their interaction with baicalein may affect the activity of ESR1. Based on the formation of hydrogen bonds, multiple hydrogen bonds were observed between baicalein and ESR1 during the simulation process. Among them, the hydroxyl group of baicalein forms stable hydrogen bonds with the amino acid residues GLU353, ARG394, etc. of ESR1. The formation of hydrogen bonds plays an important role in the stability of drug-receptor binding, as it can enhance the interaction between drugs and receptors. In addition to hydrogen bonding interactions, there is also a hydrophobic interaction between baicalein and ESR1. The aromatic ring of baicalein forms hydrophobic interactions with amino acid residues L346, R394, etc. of ESR1. Hydrophobic interactions can increase the binding affinity between drugs and receptors, enhancing the biological activity of drugs.

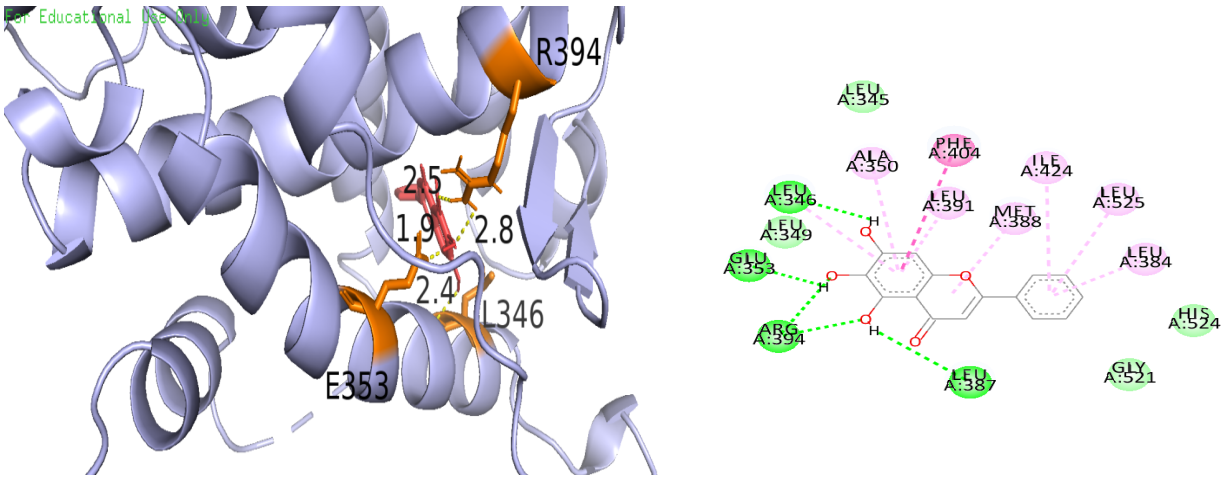

Figure 1. The effect of baicalein on the conformational changes of ESR1.

3.2. Overall conformational changes

By analyzing the trajectory files obtained from the production simulation, the study found that the overall conformation of ESR1 changed to some extent after binding with baicalein. Specifically, the LBD part of the receptor undergoes contraction, making the binding pocket more tightly packed. This conformational change may affect the interaction between ESR1 and other proteins, thereby regulating the signaling pathway of cancer cells.

3.3. Local conformational changes

In addition to the overall conformational changes, we also observed that the local conformation of ESR1 changed after binding with baicalein. For example, the side chain conformation of amino acid residue His524 has changed, making its interaction with baicalein more closely. Local conformational changes may affect the active site of ESR1, thereby regulating its biological function.

3.4. The effect of baicalein on cancer cell signaling pathway

3.4.1. Estrogen signaling pathway

ESR1 is one of the key molecules in the estrogen signaling pathway, which can bind to estrogen, activate downstream signaling pathways, and promote the growth and proliferation of cancer cells. The study found that baicalein can inhibit the binding of ESR1 to estrogen, thereby blocking the estrogen signaling pathway, after binding to ESR1. This may be one of the important mechanisms by which baicalein inhibits cancer cell growth.

3.4.2. Other signaling pathways

In addition to the estrogen signaling pathway, ESR1 can also interact with other signaling pathways to regulate the growth and proliferation of cancer cells. The study found that baicalein, when combined with ESR1, can affect the interaction between ESR1 and other signaling pathway molecules, thereby regulating the signaling pathway of cancer cells. For example, baicalein can inhibit the interaction between ESR1 and PI3K/Akt signaling pathway molecules, thereby suppressing the growth and proliferation of cancer cells.

3.5. Combining free energy analysis

By using the MM/PBSA method to calculate the binding free energy between baicalein and ESR1, the study obtained a binding free energy of -9.6 kcal/mol. The negative binding free energy indicates that the binding of baicalein to ESR1 is spontaneous and relatively stable. The magnitude of binding free energy can reflect the strength of drug-receptor binding, and the lower the binding free energy, the more stable the drug-receptor binding.

3.6. Molecular dynamics simulation

As shown in **Figure 2A**, over time, the energy exhibits significant fluctuations, sometimes rising to higher levels and sometimes falling to lower levels. This fluctuation may be caused by various interactions within the system, including intermolecular interactions, thermal motion, and other factors. It is worth noting that although the energy fluctuates greatly overall, it remains relatively stable during certain periods without significant fluctuations. This may be because the system has reached a certain equilibrium state, where the interactions between molecules are in a relatively stable stage.

Throughout the entire period, both curves showed a fluctuating upward trend, indicating significant changes in molecular structure over time. As shown in **Figure 2B**, the RMSDs curve is smoother than the Backbone curve, which means that the variation amplitude of RMSDs is smaller, while the variation amplitude of the Backbone is larger. During the period of approximately 10,000 to 20,000 ps, both curves showed significant peaks, which may be due to drastic changes in the molecular structure during this period. During the period of approximately 30,000 to 40,000 ps, both curves tend to stabilize, which may be due to the molecular structure having already stabilized.

Figure 2C shows that the RMSF of the largest amino acid residue in the docking complex is below 2.5 Å, indicating low flexibility of the complex. Calculate the binding free energy of docked complexes using the ESR1 model, where positive energy represents more favorable binding.

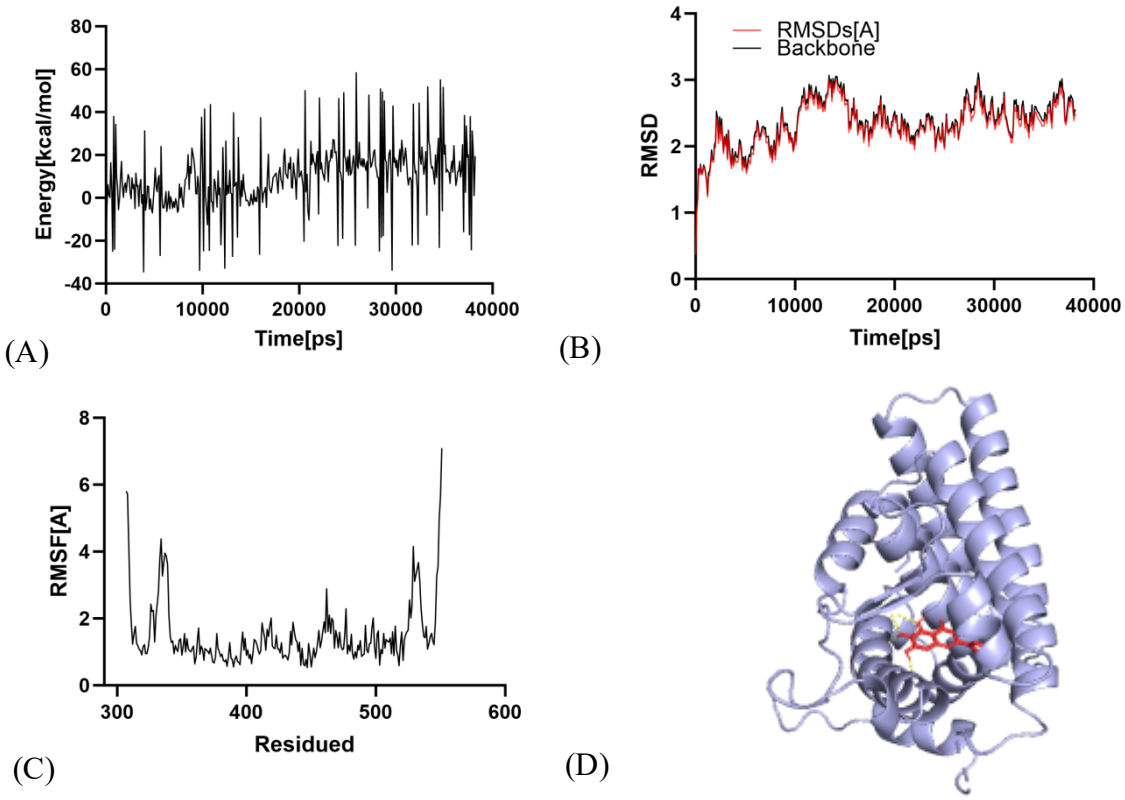

Figure 2. Molecular dynamic simulation. **(A)** ESR1-baicalein molecular dynamics modeling energy fluctuations; **(B)** ESR1-baicalein molecular dynamics simulation of root mean square deviation; **(C)** ESR1-baicalein molecular dynamics simulates atomic energy variations; **(D)** ESR1-baicalein molecular docking

4. Conclusion

This study used molecular dynamics simulation methods to explore the mechanism by which baicalin inhibits cancer cell growth through ESR1. The results, based on simulation data, provide a biological explanation of how baicalin alters the state of ESR1 to inhibit cancer cell growth. Baicalin primarily binds to the ligand-binding domain of ESR1 through hydrogen bonds and hydrophobic interactions. After binding, the conformation of ESR1 changes, affecting its interactions with other proteins and thus modulating the signaling pathways of cancer cells. Binding free energy analysis shows that the binding of baicalin to ESR1 is spontaneous and relatively stable.

This study provides a theoretical basis and experimental foundation for the potential use of baicalin as an anticancer drug. However, the study has some limitations, such as limited simulation time and simplified systems. Future research can extend the simulation time and consider more physiological factors to simulate the interactions between baicalin and ESR1 more accurately. Additionally, experimental methods can be used to validate the results of molecular dynamics simulations, providing stronger support for the clinical application of baicalin.

In summary, this study offers new insights and methods for the investigation of the anticancer mechanisms of baicalin, contributing to the development of novel anticancer drugs.

Disclosure statement

The authors declare no conflict of interest.

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