Establishment of a high-efficiency screening method for potential inhibitors of P-glycoprotein and its evaluation in vitro and in vivo

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【Summary】:Multidrug resistance (MDR) is a serious drug resistance phenomenon in tumor cells, which is an important reason for the failure of chemotherapy. P-Glycoprotein (P-gp) is the earliest discovered ABC transporter. The overexpression of P-gp in tumor cells is one of the common mechanisms of MDR, which will lead to the failure of clinical chemotherapy. The combined use of anticancer drugs and P-gp inhibitors can increase the accumulation of chemotherapeutic drugs in tumor cells, thereby improving the therapeutic effect of chemotherapeutic drugs and reversing MDR. At present, P-gp inhibitors have been developed to the third generation, and they have stopped in preclinical or clinical research due to serious toxic and side effects. Therefore, the development of P-gp inhibitors with high selectivity and low toxicity is of great significance for reversing the MDR mediated by P-gp in tumor therapy. Traditional Chinese Medicine (TCM) has the advantages of novel chemical structure and less toxicity, and is an important source of P-gp inhibitor screening. At present, the interaction research between small molecules and P-gp mainly stays at the cellular level. The experimental methods are time-consuming and labor-intensive, and the accuracy is limited. In addition, the evaluation of P-gp inhibitors is still in the in vitro stage, and there are relatively few in vivo studies. Aiming at the problems faced in the screening of P-gp inhibitors, this thesis takes P-gp as the research core, and uses a variety of membrane protein stabilization strategies to obtain P-gp proteins with correct conformation and good activity, combined with surface plasmon resonance (Surface Plasmon Resonance Resonance) , SPR) technology has the advantages of high specificity and rapidity, and established a new method for efficient screening of P-gp inhibitors and an evaluation system for the interaction between potential inhibitors and P-gp from in vitro to in vivo. The research content of this subject is mainly divided into the following three parts: 1. Establishment of a screening method for potential inhibitors of traditional Chinese medicine P-gp based on lentiviral vector membrane protein stabilization strategy combined with SPR. Lentiviral particles (LVP) stabilized membrane protein strategy and Combined with SPR technology, a P-gp specific ligand screening system was constructed to screen potential inhibitors of P-gp from TCM, and to verify its biological activity in vitro. First, lentiviral particles with different P-gp expression levels were constructed, and the expression of P-gp on the lentivirus was identified. Then, the lentiviral particles with high and low expression of P-gp were immobilized on different channels of CM5 chip for affinity detection. The positive drugs Valspodar and cyclosporine (Cyclosporine A, The K_D values for binding between Cs A) and P-gp on the chip were 14.09 μM and 16.41 μM, respectively. Using this screening system, 3 compounds were screened as potential P-gp ligands from 40 TCM monomers. Affinity experiments showed that the K_D values of honokiol, honokiol and resveratrol were 15.88, 6.44 and 70.01 μM for binding to P-gp on the chip, respectively. Subsequently, in vitro verification experiments were carried out, and the bidirectional transport experiments of Rhodamine 123 (Rhodamine 123, Rh123) showed that these three compounds could inhibit the efflux of Rh123. In addition, honokiol and resveratrol can reduce the IC_(50) value of Adr on MCF-7/ADR cells from 17.54±4.54 μg/mL to 9.56±0.60 μg/mL and 8.00±1.60 μg, respectively /mL, indicating that these two compounds have the activity of reversing MDR in MCF-7/ADR cells. Western blot (WB) and flow cytometry analysis showed that both honokiol and honokiol could down-regulate the expression level of P-gp in drug-resistant cancer cells. In this experiment, a novel P-gp ligand screening system was constructed based on lentiviral membrane protein stabilization technology combined with SPR biosensor. Using lentivirus as the carrier of P-gp, it can maintain its natural conformation. The method is highly specific and can be used for In vitro studies of small molecule interactions with P-gp. Compared with traditional cell experimental screening, the experimental time is greatly shortened. 2. The strategy of SMA polymer extraction of cell membrane proteins combined with SPR to establish a new method for rapid screening of potential P-gp inhibitors to study the use of styrene-co-maleic acid (SMA) membrane protein stabilization strategy,

the P-gp Fixed in its endogenous lipid environment, combined with SPR technology to establish a rapid screening method of P-gp potential inhibitors. First, using normal breast cancer cell MCF-7 and adriamycin (Adr)-resistant breast cancer cell MCF-7/ADR as models, the SMA polymer technology was used to extract membrane proteins from the cell membrane to obtain the corresponding protein lipids. Plasmids (SMA lipid particles, SMALPs). At the same time, the pretreatment method of SMA polymer extraction membrane protein was optimized, and the methodological investigation of the screening system was carried out, including liposome stability, chip saturation, chip specificity and the interference of SMA polymer on the screening results. The experimental results show that the optimal pretreatment condition of SMA polymer is ultrasonic disruption of cell membrane for 20 s, The reaction time was 4 h, the membrane concentration was 40 mg/mL, and the K_D value of the positive drug Valspodar combined with the chip was 26.12 μM, indicating that the chip had good specificity. The stability of liposomes in the chip was 24 h, and the residual SMA polymer in the sample did not interfere with the test results. The SMALPs were coupled to different channels of the L1 chip for affinity detection, thereby constructing a screening system for P-gp potential inhibitors (P-gp-SMALP-SPR). Using this screening system, 9 monomer components were screened from 50 kinds of Chinese medicine monomers as target compounds, and their biological activities were verified in vitro. The results showed that tetrandrine, tetrandrine, tetragonine, baicalein and icariin could significantly reduce the IC_(50) value of Adr on MCF-7/ADR cells. Tetrandrine, tetragonine and baicalein can reverse the multidrug resistance mediated by P-gp by inhibiting the function of P-gp. The screening system established in this experiment has strong specificity, which can realize rapid screening of potential P-gp inhibitors and greatly shorten the screening time. 3. In vivo evaluation of potential P-gp inhibitors for reversing tumor multidrug resistance - taking tetrandrine as an example Currently, there are few in vivo studies on potential P-gp inhibitors, and there is a lack of in vivo efficacy, drug interactions and toxicological properties data . Tetrandrine (TET) is a potential inhibitor of P-gp obtained by the P-gp-SMALP-SPR screening system. Cell activity experiments confirmed that TET can reduce the IC_(50) value of Adr on MCF-7/ADR cells. It decreased from 86.09±4.74μM to 13.52±0.63μM. Among the 9 small molecules screened, the activity of reversing MDR was the highest in vitro. To evaluate the reversal of MDR activity by TET in vivo, MCF-7/ADR cells were used to build a mouse xenograft tumor model, and the combined administration method was used to compare the weight and volume of the tumor body with TET, a potential inhibitor of P-gp, combined with paclitaxel (Paclitaxel, PTX).) in the treatment of MCF-7/ADR xenografted tumors, the tumor inhibition rate of PTX alone on tumor mice was 42.34%, the tumor inhibition rate of TET alone group was 61.03%, and the tumor inhibition rate of PTX and TET combination therapy was 42.34%. 82.44%, which is twice as much as the anticancer drug PTX alone. An LC-MS/MS method was established for the determination of PTX in plasma and tumor tissue. The effects of TET on the distribution of PTX in the plasma and tumors of mice were compared, so as to comprehensively evaluate the reversal of P-gp-mediated tumor MDR by TET. The results showed that the specificity of the LC-MS/MS method in plasma and tumor tissue was good, the intra-day and inter-day precision RSD values were less than 15%, the matrix effect RSD value was less than 15%, and the matrix had no effect on the determination of PTX. After TET combined with PTX administration, the accumulated amount of PTX in the tumor increased from 17.51±8.46 (ng/g) to 141.75±70.15 (ng/g), indicating that TET can improve the inhibition of PTX on the xenograft of drug-resistant human breast cancer in nude mice At the same time, the combination of TET does not increase the concentration of the anticancer drug PTX in the plasma. The distribution of PTX in tumors was monitored by in vivo imaging of small animals, and the results showed that TET increased the accumulation and distribution of PTX in drug-resistant tumors. In this experiment, an in vivo evaluation system for the reversal of tumor MDR activity by potential P-gp inhibitors was established from three aspects: tumor size and weight, plasma concentration of PTX, and changes in the accumulation and distribution of PTX in tumor tissue.

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