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论文

基于极化荧光方法的人LOX-1配体高通量筛选

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摘要：

目的建立以极化荧光为检测方法的高通量筛选技术，通过大规模筛选发现氧化型低密度脂蛋白受体-1(LOX-1)的天然配体。方法密度梯度超速离心获得正常人血中低密度脂蛋白(LDL)，然后用$CuSO_4$(5 μmol·L^{-1})修饰为氧化型低密度脂蛋白(oxLDL)。FITC标记hLOX-1，以受体(LOX-1)和配体(oxLDL)的相互作用为基础建立筛选模型，用极化荧光检测方法，在激发光485 nm、发射光525 nm,对3 200个样品进行高通量筛选，并用Z′因子值评价实验。结果Z′因子值为0.75，根据建立的基于极化荧光的高通量筛选实验方法，发现3个化合物与hLOX-1有较高的结合活性，IC_{50}值小于31.6 μmol·L^{-1}。结论极化荧光检测方法适合于高通量筛选技术，具有较高的稳定性、灵敏性和可重复性。

关键词： 极化荧光 高通量筛选 氧化型低密度脂蛋白受体-1 动脉粥样硬化

Identification of ligands for human LOX-1 through fluorescence polarization-based high throughput screening

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Abstract:

AimTo develop a fluorescence polarization-based high throughput screening and identify ligands for human Lectin-like oxidized low-density lipoprotein receptor-1 (hLOX-1). Methods Sequential ultracentrifugation at 4 ℃ from normolipidemic fasting volunteers to obtain low density lipoprotein (LDL), which was modified by $CuSO_4$ (5 μmol·L^{-1}) at 37 ℃ for 24 h. The assay was based on the interaction between receptor and ligand, and hLOX-1 was labeled by FITC and bound to its specific ligand, oxLDL. Different reaction time and DMSO concentration were optimized to determine the stability and tolerance of fluorescence polarization (FP) assay. 3 200 compounds were screened in black 384-well microplate by FP-based competitive displacement assay, at excitation filter of 485 nm and emission filter of 530 nm. Z′ was used to assess the assay quality. ResultsThe FP-based HTS was formatted in a 384-well microplate with a Z′ factor of 0.75, and three active compounds for hLOX-1 were identified with IC_{50} below 40 μmol·L^{-1} from total 3 200 compounds. ConclusionThe results indicated that the fluorescence polarization assay is stable, sensitive, reproducible and well suited for high throughput screening efforts.

Keywords: high throughput screening LOX-1 atherosclerosis fluorescence polarization

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1. 张天泰;杜冠华.极化荧光在高通量药物筛选中的应用[J]. 药学学报, 2005,40(4): 289-293

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