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论文

基于报告基因的雌激素受体α亚型配体筛选模型的建立和应用

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摘要：

目的 建立基于报告基因的雌激素受体α亚型配体的筛选模型，用此模型对收集到的化合物进行筛选，以发现具有新结构的雌激素受体配体。方法 构建诱导性表达载体并转染入ERα $^{+}$HepG2细胞中，筛选报告基因CAT表达受雌二醇诱导的阳性克隆。采用ELISA法检测化合物对CAT表达的影响，体外细胞存活实验对化合物进行功能性检测。结果 ERα $^{+}$HepG2细胞中CAT的表达受雌二醇的诱导并呈现剂量依赖关系。化合物三羟基二苯乙烯诱导CAT表达的最大活性为雌二醇的1.75倍。结论 利用此模型通过测定CAT基因的诱导表达水平可筛选得到新结构的雌激素受体配体。

关键词： 雌激素应答序列；报告基因；药物筛选模型 雌二醇 抗辐射损伤

ESTABLISHMENT AND ITS APPLICATION OF A REPORTER-BASED SCREENING MODEL FOR DISCOVERING NEW LIGANDS OF ESTROGEN RECEPTOR α SUBTYPES

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Abstract:

AIM To establish a sensitive and efficient reporter gene based screening model and use it to screen compounds for discovering new ligands of estrogen receptor α subtype. METHODS A recombinant Epstein-Barr virus episomal vector (pMT/ERE-CAT) was constructed by inserting a synthetic sequence composed of five estrogen response elements upstream of promoter and a chloramphenicol acetyltransferase (CAT) gene downstream of promoter. pMT/ERE-CAT was transfected into HepG2 cells expressing estrogen receptor α subtype (ER $^{+}$HepG2). Hygromycin (200 μg.mL^{-1}) was added 48 h after transfection for selection. One stably transfected clone was isolated and used to screen compounds for activity of stimulating CAT gene expression using colorimetric CAT assay. RESULTS In the ER +HepG2 cells, the expression of CAT gene was induced by estradiol. A dose-dependent expression of CAT gene with half-maximal induction at 0.07 nmol.L^{-1} was observed. The ER $^{+}$HepG2 cell was used to screen compounds for activity of stimulating CAT gene expression. Resveratrol was found to produce a maximal level of induction (1.75 times of estradiol). In vitro radiation survival experiment showed that the radiopretection activity of resveratrol (D_0 =3.18 Gy) is stronger than that of estradiol (D_0 =2.59 Gy). CONCLUSION Vector pMT/ERE-CAT was used to generate stably transfected ER $^{+}$HepG2 cell lines. The cell lines can be used to screen compounds for estrogen activity by testing extracts of cells grown in microtiter wells directly using colorimetric CAT assay. This system should provide an efficient method for screening and analyzing the activity of large numbers of ligands of estrogen receptor.

Keywords: reporter gene drug screening model estrogen antiradiation effect estrogen responsive element (ERE)

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