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实验方法

用于筛选组蛋白去乙酰化酶抑制剂细胞模型的验证

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摘要 目的 验证已建立的组蛋白去乙酰化酶(HDAC)抑制剂筛选细胞模型，并采用该模型进行药物筛选。方法 采用脂质体转染法将含有TA1和TA2启动子元件的荧光素酶报道基因真核表达载体pTA1-Luc和pTA2-Luc导入COS-7细胞，G418筛选获得稳定转染上述报告基因系统的细胞克隆。以特异性HDAC抑制剂缩酯环肽FK228，N-辛二酰苯胺异羟肟酸(SAHA)和曲古抑菌素A（TSA）为阳性对照，通过测定荧光素酶活性评估TA1和TA2启动子对HDAC抑制剂的反应；并采用此细胞模型对其他抗肿瘤药物和植物提取物进行初步筛选。结果 稳定转染的COS-pTA1及COS-pTA2细胞对HDAC抑制剂具有良好的浓度依赖性(FK228：相关系数为0.7236；SAHA：相关系数为0.7997；TSA为相关系数为0.9815；$P<0.01$)，其中COS-pTA1的特点是灵敏度高，可以有效避免因样品活性低而出现漏检。COS-pTA2细胞的特点是检测背景低，特异性强，可以有效排除假阳性的标本。两种细胞模型的联合筛选，可以鉴定具有HDAC抑制活性的化合物。结论 该细胞模型可用于筛选具有HDAC抑制活性的先导化合物。

关键词 组蛋白去乙酰化酶抑制剂 荧光素酶报道基因 细胞模型 药物筛选

分类号 R965.1，R962

Verification of cell models for screening histone deacetylase inhibitors

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Abstract

OBJECTIVE To verify cell models which can be used for screening candidates for histone deacetylase (HDAC) inhibitors. **METHODS** Two eukaryotic luciferase report vectors containing TA1 and TA2 promoters which can be activated specifically by HDAC inhibitors were constructed and named pTA1-Luc and pTA2-Luc. These plasmids were transfected into COS-7 cells, and the stable transfectants were selected by G418 and named COS-pTA1 and COS-pTA2 respectively. The responsiveness of COS-pTA1 and COS-pTA2 cells to either HDAC inhibitors 〔depsipeptide FK228, suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA)〕 or other anticancer reagents were evaluated via luciferase assay and other natural anticancer drugs were preliminarily screened. **RESULTS** The COS-pTA1 and COS-pTA2 cells showed time- and concentration-dependent response to different HDAC inhibitors by luciferase assay. The coeffienent of correlation of FK228, SAHA and TSA was 0.7236, 0.7997 and 0.9815 respectively($P<0.01$). Various HDAC inhibitors induced higher luciferase activities in COS-pTA1 cells than in COS-pTA2 cells. The data indicated that COS-pTA1 cells were more sensitive, while COS-pTA2 cells had better specificity to identify possible HDAC inhibitors. The combination of these two cell models could offer optimal potential to screen drugs with HDAC inhibitory activity. **CONCLUSION** COS-pTA1 and COS-pTA2 cell models may be useful tools for discovering lead compounds of HDAC inhibitors through library screening.

Key words histone deacetylase inhibitor luciferase reporter gene cell model drug screening

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