化学学报 2006 64 (20): 2096～2100 ISSN: 0567-7351 CN: 31-1320/06

发夹型荧光分子探针用于**DNA**甲基化酶的活性分析及其在药物筛选中的应用

周兴旺[1,2], 李军[1,2,3], 王柯敏*[,1,2,3], 谭蔚泓[1,2,3], 羊小海[1,2], 孟祥贤[2,3], 颜鸿飞[1,2]

([1]湖南大学化学生物传感与计量学国家重点实验室 湖南大学化学化工学院 长沙 410082)

([2]湖南大学生物医学工程中心 长沙 410082)

([3]湖南省生物纳米工程技术研究中心 长沙 410082)

收稿日期 2006-1-17 修回日期 2006-3-24 网络版发布日期 2006-10-27 接受日期 2006-7-27

摘要 报道了一种基于发夹型荧光探针的甲基化酶活性的分析方法,
甲基化酶和相应的限制性内切酶的识别位点被设计在发夹型探针的茎部, 四甲基罗丹明(TAMRA)
被连接在探针的5'端, 其荧光被连在3'端的熄灭基团4-(4'-二甲基对胺基偶氮苯)苯甲酸(DABCYL)所熄灭.
限制性内切酶可切割未发生甲基化修饰的探针, 导致探针的发夹结构遭到破坏, 引起TAMRA荧光信号的恢复.
根据荧光信号的恢复程度可实现对甲基化酶活性的分析. 在此基础上, 建立了一种简便、
快速分析抗肿瘤药物对DNA甲基化酶活性的影响的方法,
为筛选针对基因甲基化异常引起的恶性肿瘤的治疗药物提供了一种新的思路和方法.

关键词 分子探针 DNA甲基化酶 荧光 药物筛选

分类号

An Assay for the Activity of DNA Methylase Based on a Hairpin Fluorescence Molecular Probe and Its Application in Drug Selection

ZHOU Xing-Wang[1,2], LI Jun[1,2,3], WANG Ke-Min*[,1,2,3], TAN Wei-Hong[1,2,3], YANG Xiao-Hai[1,2], MENG Xiang-Xian[2,3], YAN Hong-Fei

([1] *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemi-cal Engineering, Hunan University, Changsha 410082*)

([2] *Biomedical Engineer-ing Center, Hunan University, Changsha 410082*)

([3] *Hunan Engineering Research Center for Bio-Nanotechnology, Changsha 410082*)

Abstract A new activity assay for DNA methylase based on a hairpin molecular probe was proposed in this paper. The recognition site of DNA methylase and corresponding restriction endonuclease was designed in the stem part of the probe, tetramethylrhodamine (TAMRA) was attached at the 5' terminus and its fluorescence was quenched by 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL) linked at the 3' terminus. The unmethylated probe can be cut in the recognition site by restriction endonuclease, causing the restoration of the fluorescence of TAMRA. Therefore, the activity of methylase would be analyzed according to the degree of fluorescence restoration. Based on this assay, the influence of anti-tumor drugs on the activity of methylase was investigated by adding drugs in the methylation reaction. This method can provide a potential in selecting proper drugs for tumors caused by altered gene methylation pattern.

Key words molecular probe DNA methylase fluorescence drug selection

DOI:

通讯作者 王柯敏 kmwang@hnu.cn