点击复制

慢病毒载体在人角质形成细胞基因组中的整合位点

工具/TOOLS
引用本文的文章/References
下载 PDF/Download PDF(695KB)
立即打印本文/Print Now
推荐给朋友/Recommend
查看/发表评论/Comments

Title: Analysis of lentiviral vector integration site preference in human keratinocytes

作者： 钱卫; 彭代智; 王丽华; 刘小玲; 李睿夫; 舒文婷; 何传果; 刘潇; 周新; 刘敬
第三军医大学西南医院全军烧伤研究所，创伤、烧伤与复合伤国家重点实验室

Author(s): Qian Wei; Peng Daizhi; Wang Lihua; Liu Xiaoling; Li Ruifu; Shu Wenting; He Chuanguo; Liu Xiao; Zhou Xin; Liu Jing
State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Burns, Southwest Hospital, Third Military Medical University, Chongqing, 40038, China

统计/STATISTICS
摘要浏览/Viewed 156
全文下载/Downloads 71
评论/Comments

RSS XML

摘要： 目的 检测慢病毒载体（lentiviral vector）在人角质形成细胞基因组中的整合位点，初步分析慢病毒载体在表皮细胞基因组中的整合位点分布规律。 方法 以本课题组前期研究中构建的慢病毒载体感染的人永生化角质形成细胞系为研究对象，应用连接介导PCR（ligation-mediated PCR,LM-PCR）技术克隆慢病毒载体在其基因组中的整合位点序列，测序克隆片段后经在线工具GTSG-QuickMap在人类基因组上定位，从而得到整合位点。再从整合位点分布与染色体、基因及其转录起始位点的关系来分析慢病毒载体在人角质形成细胞基因组中的整合倾向性。 结果 对1 148个阳性转化子DNA测序及定位分析，共得到199个整合位点。与GTSG-QuickMap模拟的随机对照相比，慢病毒载体的整合频率在第4、5、15、16号染色体上、基因转录起始位点上游5 kb至50 kb范围内显示出统计学显著性差异。 结论 慢病毒载体倾向于整合在人角质形成细胞基因组中的基因转录起始位点附近区域，而并不倾向于整合在基因内部的整合模式。

Abstract: Objective To determine lentiviral vector integration sites in human keratinocytes, and to make a preliminary analysis of lentiviral vector integration preference in human epidermal cells. Methods Ligation-mediated PCR (LM-PCR) was used to clone integration site sequences of lentiviral vector in the genome of transfected human immortalized keratinocyte line from our previous study. After the cloned DNA fragments were sequenced, the sequence reads

were inputted into the online tool GTSG-QuickMap as requested and mapped in the human genome to get the integration sites. The integration preference of lentiviral vector in human keratinocytes was analyzed in view of the relationship between integration sites distribution and chromosomes, genes and transcription start sites.　Results　A total of 1 148 positive transformants were sequenced and analyzed, and 199 unique integration sites were obtained. As compared with the matched random control simulated by GTSG-QuickMap, the integration frequencies of lentiviral vector in chromosomes 4, 5, 15 and 16 and 5~50 kb upstream region of transcription start sites showed statistically significant differences.　Conclusion　This study reveals an integration pattern that lentiviral vector preferentially integrates into the vicinity of transcription start sites with no favor for genes in the genome of human keratinocytes, and provides experimental evidences for assessing the latent risk of lentiviral vector used in gene therapy for skin diseases.

参考文献/REFERENCES

钱卫，彭代智，王丽华，等. 慢病毒载体在人角质形成细胞基因组中的整合位点分析[J].第三军医大学学报,2013,35(1):24-28.

备注/Memo:　-