中国医学科学院学报 2007 29 (3): 293-301 ISSN: 1000-503x CN: 11-2237/R

论著

转基因鼠中人α类珠蛋白基因簇染色质构象调控变化

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收稿日期 2007-1-26 修回日期 网络版发布日期 2007-7-9 接受日期

摘要 摘要：目的 用人珠蛋白转基因鼠模型建立染色质构象捕获的技术体系,
探讨人α类珠蛋白基因簇的染色质构象变化与基因表达调控的关系。方法
使人α类珠蛋白转基因纯合子公鼠与KM母鼠交配,从怀孕14.5
d的母鼠体内取出珠蛋白基因表达组织胎肝和非表达组织胎脑细胞,用甲醛交联固定细胞核内的染色质构象,
限制性内切酶NcoⅠ消化后用T4 DNA连接酶连接,解交联后提取并纯化连接的基因组DNA,
在连接位点的两侧设计引物,用半定量PCR分析胎肝和胎脑中人α类珠蛋白基因簇染色质构象模式。结果
以含HS40 的限制性酶切片段作固定片段时,
在胎脑中其他限制性酶切片段与其交联效率随线型距离增加而降低；而在胎肝中,表达的珠蛋白基因α2、α1
的酶切片段与其交联效率明显高于胎脑,
含有已关闭珠蛋白基因ζ的酶切片段与其交联效率与胎脑相当。以含α2的限制性酶切片段作固定片段时,
在胎脑中其他限制性酶切片段与其交联效率随线型距离增加而降低；而在胎肝中,上游调控元件HS 40和33
与其交联效率明显高于胎脑； HS 10和8与其交联效率略低于胎脑。结论 在表达组织中,
人α类珠蛋白基因簇上游远端调控元件通过形成染色质环与下游表达基因靠近,
从而调控α类珠蛋白基因的表达；而在非表达组织中,无此染色质环形成。

关键词 珠蛋白基因簇；转基因鼠；染色质构象

分类号

Change of the Chromosome Conformation of Human α-globin Gene Locus in Transgenic Mice

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Abstract ABSTRACT:Objective To establish chromosome conformation capture (3C) strategy and to use this method for exploring the effect of chromosome conformation on human α-globin gene expression in the human α-globin transgenic mouse. Methods Homozygous human α-globin transgenic male mouse was crossed with KM female mouse. The 14.5-day post-coitum (dpc) embryos were used for the isolation of fetal liver and fetal brain cells. Homogeneous single-cell suspension was treated with formaldehyde to crosslink the chromatin conformation in the nuclear. The cross-linked chromatin compound was digested with NcoⅠ and then ligated with T4 DNA ligase. The ligated compound was reversely cross-linked and then the ligated genomic DNA was purified for PCR analysis. The primers were designed along the two sides of cut and ligated sites. Semi-quantitative PCR was used to analyze the chromosome conformation of the whole human α-globin gene locus in fetal liver and fetal brain cells. Results When HS40 fragment was used as the fixed fragment, in fetal brain cells, the ligation frequencies of HS40 fragment with other fragments were decreased as the linear distances to HS40 fragment were increasing; while in fetal liver cells, two active genes (α1 and α2) fragments showed higher ligation frequencies with HS40 fragment than other fragments. However, the fragment containing an inactive gene (ζ) displayed the comparable low ligation frequency as that in fetal brain. When α2 fragment was used as the fixed fragment, similarly, in fetal brain cells the ligation frequencies of α2 fragment with other ones were decreased as the linear distances increasing; when in fetal liver cells, it showed higher ligation frequencies with two upstream regulatory elements (HS 40 and 33). However, it showed a little bit lower ligation frequency with another two upstream regulatory elements (HS10 and 8) than those in fetal brain. Conclusion In fetal liver cells, the distant regulatory elements are in close proximity to the downstream of the expressed globin genes through looping out the interval region; however, in fetal brain, they were not in vicinity to the expressed globin genes.

Key words globin gene cluster transgenic mice chromosome conformation

DOI:

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