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应用双色荧光原位杂交技术检测克氏综合征 The Application of Dual-color Fluorescence in situ Hybridization to the Diagnosis of Klinefelter Syndrome

刘永章1,2,吴雪昌1,金龙金2,董杰影2 LIU Yong-Zhang1,2,WU Xue-Chang1,JIN Long-Jin2,DONG Jie-Ying2

1.浙江大学生命科学学院，浙江 杭州 310012； 2.温州医学院生物教研室，浙江 温州 325027

1.College of Life Science,Zhejiang University,Hangzhou, Zhejiang 310012,China； 2.Department of Biology,Wenzhou Medical College,Wenzhou,Zhejiang 325027,China

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摘要 探讨用双色荧光原位杂交技术（dual-color fluorescence in situ hybridization,D-FISH）检测性染色体数目异常克氏综合征的应用价值，建立常规分裂期染色体和间期细胞FISH技术的实验方法。以Biotin标记的X染色体α-卫星DNA（pBamX7）探针和以Digoxigenin标记的Y染色体长臂末端重复序列（pY3.4）探针对19例克氏综合征标本同时进行外周血染色体及其间期细胞核的原位杂交，分别用Avidin-FITC和Rhodamine-FITC及其Anti-avidin进行信号的检测与放大，DAPI复染。于Olympus AX-70型荧光显微镜下，分别通过WIB、WIG及其WU滤光镜观察杂交信号及其染色体或间期核背景，并统计外周血中期染色体及其间期细胞核的杂交信号颗粒数量。在显微镜下可见以Biotin标记的pBamX7探针显示2个绿色杂交信号，以Digoxigenin标记的pY3.4探针显示1个红色杂交信号，染色体或间期核背景经DAPI复染显示蓝色；18例出现XXY杂交信号的细胞，染色体及其间期细胞核杂交平均出现率分别为95.89%和95%，明显大于正常对照标准值2.75%，证实核型为47，XXY，与染色体检测的结果一致；其余1例染色体核型检测为嵌合体，XXY杂交信号细胞出现率为92%，同时检出6.7%的XY杂交信号细胞（>正常对照标准值4.17%）。用FISH 技术检测性染色体数目异常克氏综合征具有快速、敏感度高、信号强、背景低、多色等优点，故FISH 技术在产前诊断检测领域中显示其重要的应用价值和发展前景。

Abstract:The objective of the work is to study the technique of dual-color fluorescence in situ hybridization(D-FISH) and its application value in the diagnosis of sex chromosomal count abnormality Klinefelter syndrome and establish an experimental approach to metaphase chromosome and interphase nucleus FISH technique.Biotin labeled alpha satellite X-chromosome DNA(pBamX7) probe and Digoxigenin labeled Y-chromosome long arm terminal repetitive sequence (pY3.4) probe were hybridized with pre-treated slides of peripheral blood chromosome and interphase nucleus in 19 cases of Klinefelter syndrome specimens.After being washed,the slides were treated with Avidin-FITC,Rhodamine-FITC and Anti-avidin,amplified with an additional layer and counter-stained with DAPI in an antifade solution.The hybridization signals,chromosomal or interphase nucleus settings were observed respectively with WIB,WIG and WU filters under fluorescence microscope Olympus AX-70,and the number of metaphase chromosome and interphase nucleus in the peripheral blood was counted.It was observed under the microscope that the Biotin labeled pBamX7 probe showed 2 green hybridization signals and that the Digoxigenin labeled pY3.4 probe showed 1 red hybridization signal.Chromosome or interphase nucleus counter-stained with DAPI showed blue.The average signal rate of chromosome and interphase nucleus hybridization was 95.89% and 95% respectively,significantly higher than the normal control (2.75%).Karyotype 47,XXY was confirmed,which agrees with the chromosomal findings.One case showed mosaic nuclei.XXY chromosome hybridization signal rate was 92% and XY hybridization signal rate was 6.7%,higher than the normal control rate of 4.17%.FISH is a valuable technique in diagnosing sex chromosomal count abnormality Klinefelter syndrome with the merits of fast speed,high sensitivity,strong signal,low background and multiple color.Therefore,FISH technique can find wide application and potential in prenatal diagnosis.

关键词 荧光原位杂交 克氏综合征 性染色体异常 DNA特异性探针 Key words dual-color fluorescence in situ hybridization Klinefelter syndrome sex chromosomal abnormalities DNA special probe

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