

通过微型细胞转入外源染色体的研究

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摘要 通过微型细胞与整个细胞融合的方法, 将小鼠B82HTQ2细胞(TK⁻)的三条染色体导入到小鼠PG19细胞(HGPRT⁻)中去, 被导入的X染色体使PG19细胞恢复了HGPRT功能。经过连续6个月的体外培养和小鼠体内接种实验证明, 这三条外源染色体在宿主PG19细胞里是相当稳定的。用细胞分类器进行的荧光抗体分析表明, 杂交细胞M58-1没有亲本B82HTQ2细胞特有的膜表面抗原H-2k, 这就证明了用微型细胞作为媒介物, 可以在不引入外源主要组织相容性抗原基因(MHC)的情况下, 实现基因的互补和导入新的遗传物质。本文还介绍了诱导产生B82HTQ2微核化细胞的最适宜条件、微型细胞的制备和纯化系统以及对微型细胞表面结构的扫描电镜观察结果, 并对M58-1细胞的致癌性进行了初步的讨论。

关键词

分类号

Study on Transfer of Exogenous Chromosomes by Means of Microcells

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Abstract

Microcells can be induced in B82HTQ2 cell cultures by colcemid but the treatment has to be controlled since its addition cause cells to progressively detach from flask surface until after 96 hours when a few began to remain. Concentration of 0.3 μ g/ml of colcemid for 38—40 hours at 37 $^{\circ}$ C gave optimal condition for producing large number of viable micro-cells in B82HTQ2 cell line. The finding of a few microcells containing one, two or a small group of chromosomes provides, a cytological proof as to the nuclear contents of microcells and supports the microspectrophotometer DNA determination of Ege and Ringertz (1976) in which DNA values equivalent to a content of 1 or 2 chromosomes were recorded.

Fine details of the microcells surface were clearly observed by scanning electron microscopy, they were seen to be totally enveloped by an intact cytoplasmic membrane with finger-like protuberances or microvilli similar to those found in intact cells.

The mean chromosome number of the parent cell lines was found to be 38 for PG19 and for B82HTQ2, whereas that in microcell-mediated hybrid M58-1 was 41 and for PG19/B82HTQ2 whole cell fusion it was 88, the sum of the two. G-banding of M58-1 cells revealed that by way of a microcell fusion, three chromosomes from a B82HTQ2 cell were introduced into a PG19 cell. The transferred X chromosome from B82HTQ2 cell restored a positive HGPRT status.

Even after six months in continuous subculture in MEM, the hybrid cells retained its mean chromosome number and moreover in the G-banding sample analysed, the transfer-red chromosomes were found to be present in very high proportion of these cells. They were also retained in six out of nine tumors induced.

The results from analysis of H-2k show that in common with PG19 cells the hybrid cells do not carry the H-2k antigen which is found in B82HTQ2 cells. In view of the results obtained, the original B82HTQ2 cell did not contribute any copy of this chromosome to the hybrid. It was demonstrated that the potential of introducing small amounts of an intraspecific genome into a cell without transfer of the genes coding for histocompatibility antigen. The method could be proved useful for correction of genetic defects, the studies concerned with graft acceptance.

Key words

扩展功能

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