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论著

人卵巢癌细胞及小鼠MTX抗性细胞中DMs大小的测定

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摘 要 目的 测定肿瘤细胞及耐药性细胞中双微体（Double minutes，DMs）的大小。方法 应用PFGE （Pulsed field gradient gel electrophoresis）与Southern杂交技术，对人卵巢癌细胞UACC-1598及氨甲喋呤（Methotrexate，MTX）抗性的小鼠胚胎成纤维细胞中DMs的大小进行检测。结果 发现UACC-1598细胞中存在2.8 Mb、2.1 Mb及1.4 Mb的DMs群体，提示多拷贝的扩增基因及其邻近的染色体区域经染色体断裂、易位及重排形成较大的DMs。同时，用浓度逐渐增高的MTX对小鼠胚胎成纤维细胞进行体外诱导，分离出富含DMs的不同MTX抗性程度的细胞群体。在MTX抗性细胞中检测到2.5 Mb及1.4 Mb的DMs群体， MTX100细胞中以1.4 Mb的DMs群体为主，而MTX500细胞中以2.5 Mb的DMs群体为主。 结论 MTX细胞中产生的扩增子结构在演化过程中趋向于寡聚化或多聚化形成较大片段的DMs。

关键词 基因扩增 双微体 PFGE Southern blot

分类号

The Sizes of Double Minutes in Human Ovarian Cancer Cells and Mouse MTX-resistant cells，Determined by Pulsed Field Gradient Gel Electrophoresis and Southern Blot

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Abstract Objective To determine the size of Double minutes (DMs), one of the predominant amplification structures found in primary tumors and drug-resistant cells. Methods PFGE (Pulsed field gradient gel electrophoresis) combined with Southern blot hybridization was used to determine the sizes of DMs in human ovarian cancer cells UACC-1598 and MTX-resistant cell derived from mouse fibroblasts 3T3R500. Results The heterogeneous DMs populations of 2.8 Mb, 2.1 Mb and 1.4 Mb in size were detected in UACC-1598 cells, which indicate that multiple copies of amplified genes and surrounding regions underwent chromosomal breakage, translocation and rearrangement to form the larger DMs. On the other hand, MTX- resistant mouse fibroblasts were isolated with stepwise increasing concentrations of MTX. PFGE and Southern blot analyses detected 2.5 Mb and 1.4 Mb DMs populations containing DHFR gene in the cells resistant to the different concentrations of MTX. The 1.4 Mb DMs is the predominant population in MTX100 cells, whereas the 2.5 Mb DMs is the predominant population in MTX500 cells. Conclusion These data suggest the initial small amplicons with the tendency of multimerization to form larger DMs during the progression. However, no DMs integrate into chromosome was observed during the course of our study.

Key words Gene amplification DMs PFGE Southern blot

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