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基础研究

胰腺癌细胞膜平衡型核苷转运载体和集中型核苷转运载体的检测

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摘要：

目的：检测胰腺癌细胞膜上Na+非依赖的平衡型核苷转运载体（ENT）和Na+依赖性的集中型核苷转运载体（CNT），并对ENT进行定量检测。方法：将胰腺癌细胞株（Panc-1）在含潘生丁（100 μmol/L）的培养基中分别温育8，15，60，120 min，收集细胞用乙腈裂解，荧光分光光度计测定细胞悬液中潘生丁的荧光强度，依据平行悬液中的细胞数量计算出单个细胞表面ENT的数量；再将Panc-1分别在含5-氟尿嘧啶（5-FU）和5-FU+潘生丁两种培养基中分别温育15，30，60，120，240 min，毛细管区带电泳法测定5-FU含量；根据平行悬液中的细胞数量和单细胞体积计算单细胞中5-FU的浓度，间接判定细胞膜上是否存在CNT载体。结果：Panc-1在含潘生丁的培养基温育8 min后即能检测到细胞的荧光强度，15 min达高峰20.2×10-19 mol；借助潘生丁测得单个细胞表面有1.25×105个ENT。用潘生丁阻断ENT后，5-FU仍可被转运入胰腺癌细胞中，且细胞内的5-FU最高浓度达到（138.3±9.77）mg/L，明显高于培养基中5-FU的浓度（P=0.011）。结论：与潘生丁结合后，通过荧光法可以定量检测细胞表面的ENT；通过阻断ENT并测定细胞中5-FU浓度，可以判断胰腺癌细胞膜上存在CNT，从而对合理应用化疗药物，提高化疗效果提供依据。

关键词： 胰腺肿瘤/药物疗法；人胰腺癌细胞株；核苷载体/药理学

Detection of equilibrative nucleoside transporter and concentrative nucleoside transporter on pancreatic cancer cell membrane

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Abstract:

Objective: To detect the sodium-independent equilibrative nucleoside transporter (ENT) and sodium-dependent concentrative nucleoside transporter (CNT) on pancreatic cancer cell membrane and make a quantitative assessment of ENT. Methods: Pancreatic cancer cell line (Panc-1) was incubated in the medium containing dipyridamole (100 μmol/L) for 8, 15, 60 and 120 min, respectively. Afterwards, the cells were harvested and lysed with acetonitrile, the fluorescence intensity of dipyridamole in the cell suspension was detected by spectrofluorometer, and the sum of ENTs on a single cell was calculated according to the number of the cells in the parallel suspension. Next, Panc-1 cells were incubated in the medium containing 5-fluorouracil (5-FU) or 5-FU plus dipyridamole for 15, 30, 60, 120 and 240 min, respectively, and the content of 5-FU in the cell was measured by capillary zone electrophoresis; the 5-FU concentration in a single cell was counted according to the number of cells and the single cell volume in the parallel suspension to indirectly determine whether CNT was present on the cell membrane of Panc-1. Results: The fluorescence was detected in Panc-1 after 8 min of incubation in the medium containing dipyridamole with a maximal level of 20.2×10－19 mol at 15 min. The sum of ENTs was determined at 1.25×105 in a single cell according to the dipyridamole content. 5-FU was still transported into the Panc-1 cells after the blockage of ENT with dipyridamole and the maximal level of intracellular 5-FU reached (138.3±9.77) mg/L, which was significantly higher than that of the medium (P=0.011). Conclusion: ENTs on the cell surface can be quantitatively determined through fluorescence intensity detection after their binding with dipyridamole. Whether CNT is present on the cell membrane can be determined by measuring the intracellular 5-FU concentration after the blockage of ENT, and therefore provides a reference for the rational use of chemotherapeutic drugs and improvement of their effect.

Keywords: Pancreatic Neoplasms/drug ther Human Pancreatic Carcinoma Cell Nucleoside Transporter/pharm

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