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实验方法

高通量实时荧光定量RT-PCR方法验证FL细胞对苯并(a)芘7,8-二氢二醇9,10-环氧化物处理的差异表达基因

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摘要 目的 验证由寡核苷酸微阵列检出的FL细胞对苯并(a)芘7,8-二氢二醇9,10-环氧化物(BPDE) 处理的差异表达基因。方法 FL细胞分别经0.1%二甲亚砜（DMSO）和0.5 μmol·L^{-1} BPDE处理，以高通量实时荧光定量RT-PCR方法(TaqMan®低密度芯片)平行检测185个目标基因的相对表达水平。结果 BPDE处理组相对DMSO对照组，51个基因表达有差异，12个基因表达上调，39个基因表达下调，（n=3，P<0.05），涉及细胞增殖，分化，凋亡调控基因，转录调节基因和代谢酶基因等。结论 高通量实时荧光定量RT-PCR可迅速对微阵列数据进行验证，差异表达谱有助于研究BPDE的毒理机制和细胞的应答反应。

关键词 RT-PCR 低密度芯片 苯并(a)芘7,8-二氢二醇9,10-环氧化物 差异基因表达

分类号 R965.2

Differential gene expression profiles in benzo(a)pyrene 7,8-dihydrodiol 9,10-epoxide treated FL cells validated by high-throughput quantitative RT-PCR assay

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Abstract

AIM To validate the differential gene expression profile of 185 target genes selected from a preliminary microarray study. METHODS The relative mRNA level in two groups of cell samples, *ie*, 0.5 μmol·L^{-1} benzo(a)pyrene 7,8-dihydrodiol 9,10-epoxide (BPDE) and 0.1% DMSO solvent treated, respectively, was evaluated by a high-throughput quantitative real-time RT-PCR approach based on the TaqMan®Low Density Array (Applied Biosystems). RESULTS The total of 51 genes (12 up-regulated and 39 down-regulated) related to cellular proliferation, differentiation,apoptosis, transcriptional regulation and metabolism etc were detected differentially expressed (n=3, P<0.05). CONCLUSION High throughput quantitative real time RT-PCR is a timesaving technique for validating the data obtained from microarray experiments. The validating results indicate that the activation of multiple signaling pathways and alteration of gene expression are responsible for the cell cycle arrest and metabolic alteration induced by BPDE exposure.

Key words RT-PCR low density array benzo(a)pyrene 7 8-dihydrodiol 9 10- epoxide differential gene expression

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