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论著

EGFR/HER2基因敲减对SPC-A-1细胞株细胞生物学特性及相关信号通路的影响

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摘要 目的： 探讨EGFR/HER2基因沉默对非小细胞肺癌细胞株EGFR酪氨酸激酶信号转导通路的交互影响及其与细胞增殖、凋亡和周期改变的关系。方法： 设计并合成EGFR、HER2及 EGFR/HER2共干扰序列，构建含干扰序列的载体，进行瞬时转染；应用实时荧光定量、蛋白印迹法检测基因沉默效果；用四甲基偶氮唑盐比色法、流式细胞仪检测基因沉默后生物学特性改变；蛋白印迹法检测EGFR下游信号通路蛋白Akt、p-Akt、p-Erk1/2、p-p38表达水平变化；采用SPSS13.0软件分析结果。结果： 在SPC-A-1细胞系中，EGFR干扰组、HER2干扰组、EGFR联合HER2干扰组和EGFR-HER2共干扰组体外细胞增殖率均有下降趋势；除EGFR干扰外，其余各组均可诱发凋亡；各组的细胞周期G1期和S期细胞比例有显著改变；基因沉默效果检测显示EGFR和HER2基因蛋白水平被下调。下游信号通路蛋白检测示EGFR下游信号通路蛋白Akt、p-Akt、p-Erk1/2、p-p38表达水平和细胞增殖、凋亡以及细胞周期改变之间未发现明显相关规律。结论： 单纯EGFR干扰SPC-A-1细胞不能诱发显著凋亡，HER2和EGFR/HER2基因共沉默后诱发的人肺腺癌SPC-A-1细胞凋亡比阴性对照显著增加。EGFR/HER2基因沉默后诱发的细胞增殖、凋亡和细胞周期改变与EGFR家族下游信号通路蛋白之间未发现显著相关关系。

关键词 癌,非小细胞肺 RNA干扰 受体,表皮生长因子

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Relationship between EGFR/HER2 gene knocked down and the downstream signal pathway in SPC-A-1

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Abstract

<FONT face=Verdana>AIM: RNAi technique was applied to explore the relationship between downstream signal pathway in EGFR family and the cell proliferation, cell cycle alteration, apoptosis after EGFR/HER2 RNA interference. METHODS: Sequence-specific siRNA for EGFR and HER2 were designed as literatures described. “Tuschl rules” and BLAST on the full length of EGFR/HER2 cDNA were used to ensure the sequence-specific siRNA for EGFR/HER2 joint interference. Based on the above sequence-specific siRNA, recombinant plasmids with GFP and neomycin resistance marker were constructed. Six groups including mock, negative control, shRNA-EGFR, shRNA-HER2, shRNA-EGFR/HER2 and shRNA-EGFR+shRNA-HER2 were established by transient transfection. Real time quantitative RT-PCR was used to detect the silencing of the EGFR/HER2 gene level. Western blotting was used to measure the levels of EGFR/HER2 protein and protein phosphorylation expression. Transfected cells were stimulated with EGF 15 min before protein extraction. MTT assay and flow cytometry were used to evaluate the cell proliferation, apoptosis and cell cycle distribution after RNAi. The protein expression levels of downstream signaling pathway proteins including Akt, p-Akt, p-Erk1/2, p-p38 were measured by real time quantitative RT-PCR and Western blotting. Randomized block analysis of variance and SNK methods were used to compare the differences between groups. RESULTS: Cell proliferation was inhibited in the groups of shRNA-EGFR, shRNA-HER2, shRNA-EGFR+shRNA-HER2 and shRNA-EGFR/HER by MTT assay. Cell cycle analysis by flow cytometry showed that apoptosis ratio in shRNA-HER2 (P<0.01), shRNA-EGFR/HER2 (P<0.01) and shRNA-EGFR+shRNA-HER2 (P<0.05) groups were significantly higher than those in negative control group, while there was no statistical difference between shRNA-EGFR and negative control (P>0.05), and that the distributions in phase G1 and phase S in shRNA-EGFR (P<0.01), shRNA-HER2

(P<0.01), shRNA-EGFR/HER2 (P<0.01) and shRNA-EGFR+shRNA-HER2 (P<0.01) were significantly different compared with the negative control. The level of EGFR/HER2 protein and protein phosphorylation expression were down regulated. The cell proliferation, apoptosis and cell cycle alterations induced by EGFR/HER2 RNA interference showed no significant relationship with downstream signal pathway molecular in EGFR family. CONCLUSION: EGFR gene knockdown may not cause significant apoptosis in SPC-A-1 cell line. The variations of cell proliferation, apoptosis and cell cycle alterations induced by EGFR/HER2 RNA interference were not found to have significant relationship with downstream signal pathway molecules in EGFR family.</FONT>

Key words Carcinoma non-small-cell lung RNA interference Receptors epidermal growth factor

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