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PKM2基因沉默对人肺癌A549细胞放射敏感性的影响

Blockage of PKM2 expression by gene silencing enhances the radiosensitivity of human lung cancer A549 cells

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中文摘要:

目的 探讨通过siRNA干扰技术沉默丙酮酸激酶同工酶M2(pyruvate kinase M2,PKM2)基因表达对人肺癌A549细胞放射敏感性的影响。方法 以人肺癌A549细胞为研究对象,根据PKM2 mRNA编码序列设计并合成干扰siRNA序列,瞬时转染A549细胞。用RT-PCR和Western blot法分别从mRNA水平和蛋白水平检测PKM2基因的表达。设PKM2 siRNA干扰组,阴性对照组、空白对照组。各组细胞分别接受不同剂量6 MV X射线照射,通过集落形成实验检测细胞放射敏感性,流式细胞仪检测各组细胞周期及凋亡率。每组实验重复3次。结果 RT-PCR和Western blot显示,PKM2 siRNA干扰组较空白对照组的PKM2 mRNA和蛋白表达都明显下降(t=20.91、47.00,P<0.01),抑制率分别为(70.27±1.38)%和(70.42±1.18)%;集落形成实验显示,PKM2 siRNA干扰+IR组A549细胞D_0、D_q、N和SF_2值均低于空白对照+IR组(t=43.82、28.44、15.60、29.63,P<0.01),放射增敏比(SER)为1.27。流式细胞仪分析显示,PKM2 siRNA干扰组G_2/M期细胞的百分率明显高于空白对照组(t=8.35,P<0.01),经10 Gy X射线照射,G_2/M期细胞比例也明显升高(t=27.87,P<0.01)。两者联合使用后G_2/M期阻滞更加明显。siRNA干扰组细胞凋亡率较空白对照组无显著升高,空白对照+IR组较空白对照组凋亡率明显升高(t=23.99,P<0.01);PKM2 SiRNA干扰+IR组较空白对照+IR组和PKM2 siRNA干扰组差异均有统计学意义(t=9.42、65.21,P<0.01)。结论 特异性沉默PKM2基因表达能够增加A549细胞的放射敏感性,推测其机制可能与改变细胞周期分布及使射线诱导细胞凋亡的作用增强有关。

英文摘要:

Objective To explore the role of pyruvate kinase M2(PKM2) siRNA in the radiosensitivity of human lung cancer A549 cells. **Methods** PKM2 siRNA was synthesized according to the coding sequence of PKM2 mRNA and then was transferred into A549 cells with lipofectamine.The expressions of PKM2 gene and protein was detected by RT-PCR and Western blot, respectively. The experiments were divided into PKM2 siRNA interference group, siRNA negative control group, and blank control group. The cells of each group were exposure to 6 MV X-rays in different dose. Radiosensitivity was evaluated by colony formation assay. Flow cytometry was applied to analyze cell cycle distribution and apoptosis. Data are representative of three independent experiments. **Results** Ccompared with blank control cells, the expressions of PKM2 gene and protein in the PKM2 siRNA transferred A549 cell was efficiently diminished (t=20.91, 47.00, P<0.01) with inhibition rates of (70.27±1.38)% and (70.42±1.18)%,respectively.Compared with control, PKM2 siRNA transfection significantly decreased the D_0, D_q, N and SF_2 values (t=43.82,28.44,15.60,29.63,P<0.01) and hence yield a sensitization enhancement ratio (SER) of 1.27. In addition, the percentage of G_2/M phase cells in the siRNA group and irradiated group were both significantly higher than that of the blank control group (t=8.35,27.87,P<0.01).The combined treatments of PKM2 siRNA interference and irradiation arrested more cells in the G_2/M phase compared to either treatment alone. The apoptosis rate of siRNA group was not dramatically different from that of blank control group.The apoptosis rate of irradiation group was higher than that of blank control group (t=23.99,P<0.01), and the combined treatments of siRNA and irradiation enhanced the apoptotic rate compared to either treatment alone (t=9.42, 65.21, P<0.01). **Conclusions** Specific blockage of PKM2 expression by gene silencing could enhance the sensitivity of human lung cancer A549 cells to radiotherapy *in vitro*,which may due to the cell cycle arrest and apoptosis induction after irradiation.

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