

替莫唑胺联合HSV1-tk/ GCV系统治疗人脑胶质瘤细胞的实验

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Combining Temozolomide with HSV1-tk/GCV Treat Human Malignant Glioma

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摘要 目的探讨替莫唑胺联合HSV1-tk/GCV自杀基因系统对人胶质瘤细胞的体外杀伤作用及其作用机制。方法用携带tk基因的重组逆转录病毒转染人胶质瘤细胞系U251细胞,并筛选、鉴定。转染与未转染tk基因的U251细胞按1:9混合。实验分3组:对照组、GCV组、GCV+TMZ组。GCV组以5种不同浓度(2、5、10、20、40 μ M)作用于混合细胞;GCV+TMZ组在上述基础上各加入TMZ 50 μ M;对照组细胞不做任何处理。各组细胞培养72h后,MTT法检测各组细胞的活力,流式细胞仪检测细胞凋亡及细胞周期分布的变化。结果(1)U251/tk细胞的活力随GCV组浓度的增加逐渐减弱,呈现良好的剂量效应关系;(2)GCV组、TMZ +GCV组的IC50分别为17.3 μ M、8.1 μ M(两组相差2.14倍);(3)GCV+TMZ组的总体抑制率显著高于GCV组($P < 0.01$)。GCV+TMZ组生存曲线明显左移;(4)流式细胞仪检测显示两组的凋亡率均明显增加($P < 0.01$);细胞多被阻滞于G2~M期。结论HSV1-tk/GCV自杀基因系统有一定的肿瘤杀伤效应及旁观者效应;替莫唑胺与HSV1-tk/GCV自杀基因系统两者之间有明显的协同作用;其作用机制可能通过改变细胞周期的分布及促凋亡增加GCV的旁观效应。

关键词: 替莫唑胺 HSV1 tk/GCV 胶质瘤

Abstract: Objective To determine whether HSV1-tk/GCV combining with temozolomide (TMZ) would enhance antitumor effect in malignant glioma in vitro. Methods Human U251 glioblastoma cells were transfected with the recombinant retrovirus which contained HSV1-tk gene. After Screening and identifying the positive ones, the transfected and non transfected cells were mixed at a 1 : 9 ratio. The cells were incubated in the presence of GCV (2,5,10,20,40 μ M), both GCV(2,5,10,20,40 μ M) and 50 μ M TMZ, or non drug for 72h respectively. Cell viability was accessed by MTT. Cell cycle progression and apoptosis ratio was determined by using flow cytometry. Results The ratio of the OD570 was significant different between the GCV group ($\geq 5\mu$ M), GCV+TMZ group ($\geq 2\mu$ M) and untreated control group respectively ($P < 0.01$). The survival curve of GCV+TMZ group was significantly shifted to the left compare with the GCV group. The GCV IC50 of two groups were 17.3 μ M (GCV group) and 8.1 μ M (GCV+TMZ group) respectively ($P < 0.01$). The flow cytometry showed that apoptosis of two groups increased significantly ($P < 0.01$) and cells were arrested in G2~M phases. Conclusion HSV1-tk /GCV suicide gene therapy system has a powerful antitumor effect and a bystander effect for glioma in vitro. It has a synergistic anticancer effect when combined with TMZ, And GCV can induce the cells apoptosis.

Key words: Temozolomide HSV1-tk/GCV Glioma

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