Experimental study on human pulmonary adenocarcinoma cell A 549 transfected with HSV-1-TK gene in vitro and in vivo  
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Abstract & Objective To observe killing effect of HSV-1-TK/GCV system on human pulmonary adenocarcinoma cell A 549 in vitro and in vivo. Methods A retroviral vector containing TK gene was constructed and transduced into pulmonary adenocarcinoma cell A 549 by electroporation. The sensitivity of transfected cell to GCV in vitro and bystander effect and cellular apoptosis were observed. The recombination and expression of TK gene were examined by DNA PCR and in situ hybridization individually. In addition the therapeutic effect of GCV on subcutaneous tumors inoculated with transfected and parental cells respectively was observed. Results The transfected cells were irregular in shape polygonal and easy of vacuolization. The double time of A 549 was 36.15 ± 3.27 hours respectively E-P > 0.05 E-Comparing the sensitivity of transfected cells to GCV was 46 times higher than that of parental cells and bystander effect was more apparent in high density inoculation cells than in low density. Apoptotic bodies and semimon feature in nuclear were observed in transfected cells but not in parental cell. Apoptotic cells were found significantly more in transfected cells than in parental cells by FCM and TUNEL E-P < 0.001 E-The recombination and expression of TK gene were positive in the transfected cells. In vivo E-growth of tumors which formed by transfected cells was significantly inhibited by GCV E-However, there was no similar inhibitive effect found in control group. Conclusion The transfected cells have obtained sensitivity to GCV. The killing effect of TK/GCV system on tumor cells is probably related to apoptosis. GCV could inhibit growth of tumors inoculated by transfected cells.

Keywords HSV-1-TK gene Gene transfer Gene therapy Lung neoplasms Cell apoptosis simplex virus- I type thymidine kinase gene HSV-1-TK enzyme

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Fig 1  Curves of growth inhibition of GCV A549, A549-PLXSN and A549-TK cells

Fig 2  Apoptotic change of A549-TK cell after three-day treatment with 50 μmol/L GCV. Arrows showed apoptotic bodies and semimoon feature $E - 10^3$.

Fig 3  Analysis of DNA PCR products from A549-TK and control cell. 1, A549-PLXSN, 2, A549-TK, 3, A549-TK, 4, A549-PLXSN.

Fig 4  mRNA expression of TK gene in transfected cell by in situ hybridization

2.2 TK $\rightarrow$ GCV $\rightarrow$ DNA $\rightarrow$ TK $\rightarrow$ 681 bp 1.00 AE $\rightarrow$ FF $\rightarrow$ TK $\rightarrow$ 1/43 AE $\rightarrow$ 667 bp $\rightarrow$ TK

2.3 $\rightarrow$ AUE $\rightarrow$ A+ GCV $\rightarrow$ AUE $\rightarrow$ TK $\rightarrow$ 1,000 bp $\rightarrow$ TK mRNA $\rightarrow$ TK mRNA $\rightarrow$ TK $\rightarrow$ 224 ± 151 bp $\rightarrow$ 167 bp $\rightarrow$ TK $\rightarrow$ TK

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50 μmol/L GCV $\rightarrow$ A311 $\rightarrow$ A549-TK $\rightarrow$ 0 μmol/24 h $\rightarrow$ 0 μmol/10 h

681 bp 654 bp 651 bp

1/42 A549-TK $\rightarrow$ GCV $\rightarrow$ DNA $\rightarrow$ TK $\rightarrow$ 681 bp $\rightarrow$ TK $\rightarrow$ 1,000 bp $\rightarrow$ TK $\rightarrow$ TK

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