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EFFECTS OF DIFFERENT CONCENTRATIONS OF ANTISENSE OLIGODEOXYNUCLEOTIDES(ASODN) ON HUMAN PANCREATIC CANCER CELL LINE PaTu8988s

CAI Jian-ting(蔡建庭), CHEN Peng(陈　鹏)
ZHONG Dan-dan(钟丹丹), QIAN Ke-da(钱可大)

(*Department of Gastroenterology, Second Affiliated Hospital, Collge of Medicine, Zhejiang University, Hangzhou 310009, China*)

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Abstract: Objective: To study the effects of different concentrations of antisense oligodeoxynucleotides (ASODN) on human pancreatic cancer cell line PaTu8988s. Method: Human pancreatic cancer cell line PaTu8988s in exponential growth stage was used to study the effect of different drug concentrations on the cell line in the presence of different concentrations (0 μg/ml, 5 μg/ml, 25 μg/ml, 50 μg/ml, 10 μg/m, 200 μg/ml) of ASODN and sense oligodeoxynucleotides (SODN). Cell counts and 3-[4,5-dimethylthiazolzyl]-2,5-diphenyl tetrazolium bromide (MTT) assays were carried out. Results: The inhibitory rate on the cell line PaTu8988s was 98.73%, 95.76%, 69.49%, 33.05% and 0 for ASODM concentrations of 200 μg/ml, 100 μg/ml, 50 μg/ml, 25 μg/ml and 5 μg/ml at 48 hours. Conclusions: K-ras complementary ASODN can inhibit the growth of human pancreatic cancer cell line PaTu8988s by 30.05% to 98.73%. This is likely to contribute to the specificity of the K-ras mRNA complementary capped ASODN sequential codon. Non-specific effect and side effect of ASOND were observed for instance, the greater the concentration is, the earlier the peak of inhibitory rate is reached. In concentration of 25 μg/ml to 100 μg/ml ASODN showed a dose-effect correlation.

Key words: pancreatic carcinoma, antisense oligodeoxynucleotides, gene therapy, K-ras, concentration.
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INTRODUCTION

Antisense oligodeoxynucleotides (ASODN) can inhibit transcription and interfere with special gene expression. A fixed point on the K-ras gene with high mutation rate in pancreatic carcinoma offers an intracellular target for pancreatic carcinoma gene therapy with ASODN (Cai et al., 2000; Duroux et al., 1995; Van lathem et al., 1998). We investigated the inhibitory effects of different concentrations of synthetic K-ras complementary ASODN on human pancreatic cancer cell line PaTu8988s. The present study may provide empirical basis for treatment of pancreatic carcinoma with ASODN in animal experiments and clinical trials.

MATERIALS AND METHODS

Materials

Human pancreatic cancer cell line PaTu8988s was kindly provided by Professor XU Guoming (Changhai Hospital, Shanghai). RPMI-1640 and IMDM medium (GIBCO BRI) were obtained from LIFE TECHNOLIGIES CO. (No. 1009040 and No. 1006255, U.S.A.). Super newborn calf serum was purchased from SIJIQING BIOTECHNOLOGY MATERIAL RESEARCH INSTITUTE (No. 80224, Hangzhou, China). Protease was from DIFCO CO. (U.S.A.). 24-well plate and 96-well microplate were from NUNC CO. (Denmark). K-ras ASODN (sequence: 5' > ACAAGTTTATATTCAGTCAT < 3') and SOND (sequence: 5' > ATGACTGAATATAAACTTGT < 3') solution were synthesized by SINGO.(Shanghai). 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from FLUKA CHEMIE AG CO. (Switzerland). ELISA-reader was from EASTERN CHINA ELECTRONIC WACUUM TUBE FACTORY.

Cell cuture

The human pancreatic carcinoma cell line

PaTu8988s was grown in RPME-1640 or IMDM medium(containing 15% heated-inactivated special grade newborn calf serum, penicillin and streptomycin) incubated at 37℃ in 5% CO_2 and subcultured. The cells in exponential growth phase after secondary culture were digested with 0.25% trypsinase, washed twice with Hank's solution and then used to make single-cell suspension.

Oligodeoxynucleotides preparation

ASODN and SODN solutions were diluted to 1mg/ml with sterilized normal saline, filtrated to sterile conditions, separated and stored at −20℃.

Ihibition of different concentrations of ASODN and SODN on the cell line PaTu8988s.

The PaTu8988s cells were harvested during the exponential growth phase. A monocellular suspension(cell concentration: 5×10^5 ml) was prepared with RPMI-1640 medium, the suspension was seeded onto a 24-well plate (0.5ml per well). Then the 5 μg/ml, 25 μgl/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml solutions of ASODN and SODN were added to the suspension respectively. Each well was doubled simultaneously and cultured at 37℃ in 5% CO_2 for 48 hours.

MTT assay

MTT assay was done as follows: After the supernatant was discarded. 0.5 ml RPMI-1640 medium containing MTT (0.2 mg/ml) was put into each well for incubation at 37℃ in 5% CO_2 for 48 hours. The supernatant was discarded, and after cosolubilization buffer (0.2 ml per well) was added to it, the solution was transferred to a new 96-well microplate. The absorption ratio of each well was read by ELISA-reader. The half inhibition concentration (IC50) was calculated. The study group and the control group were duplicated.

Chi-square test was used for statistical analysis

RESULTS

The inhibition rates of different concentrations of ASODN and SODN in human pancreatic cancer cell line PaTu8988s are shown in Table 1 and Fig.1.

Table 1 Effect of ASODN and SODN on the cell line PaTu 8988s(MTT ASSAY)

Group		Concentration(μg/ml)				
		200	100	50	25	5
ASODN	Extinction	0.015	0.05	0.36	0.79	1.19
	Inhibitory rate	98.73%*	95.76%*	69.49%*	33.05%	0
SODN	Extinction	0.905	1.06	1.18	1.205	1.20
	Inhibitory rate	23.31%	10.17%	0	0	0

* $P \leqslant 0.001$

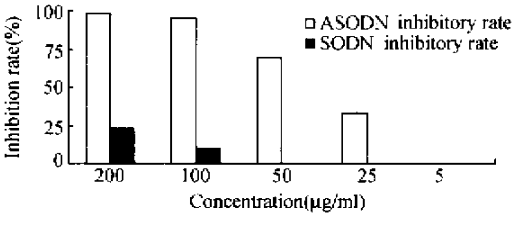

Fig.1 The inhibition rates of ASODN and SODN in the cell line PaTu 8988s

DISCUSSION

ASODN can interfere with the expression of the same gene, but has no inhibitory effects on other genes. This study showed that K-ras complementary ASODN(≥25 μg/ml) can inhibit the growth of the cell line PaTu8988s obviously by 33.05% to 98.73%, but that SODN does not inhibit the growth of PaTu8988s cell line. Comparison between antisense and sense ODN showing that ASODN could inhibit the proliferation of the cell line PaTu8988s is likely associated with the high specificity of the K-ras complementary ASODN mRNA cap (sequence: 5' > ACAAGTTTATATTCAGTCAT < 3'). Some experts found that different kinds of ASOND have different inhibition effects. For example, ASODN modificated by methyl phosphate or amide phosphate has greater inhibition effect

than normal ASODN (Iversen et al., 1994).

The ASODN concentration-effect curve shows that too low concentration leads to minimal inhibition effect. If the concentration was too high, non-specific effects and side effects of ASODN were observed. The concentration of ASODN had great influence on its effect. Initially PC-2 cells were treated with 20-mer ASODN (sequence: 5' > CCTACGCCACCAGCTCCAAC < 3') near codon 12 of c-K-ras in non-specific oligodeoxynucleotides (sequence: 5' > CCAGAGGTAAGTGGACTT < 3') study group and control group. The inhibition effect of ASODN was associated with its dose .The larger the dose was, the greater the inhibition effect was (Liu et al., 1993). This study showed that the inhibition rates on the cell line PaTu8988s were 98.73%, 95.76%, 69.49%, 33.05% and 0 for ASODN concentrations of 200 μg/ml, 100 μg/ml, 50 μg/ml, 25 μg/ml and 5 μg/ml respectively, at 48 hours. Inhibition effects of ASODN were observed only when drug concentration was higher than 25 μg/ml. And the higher the concentration of ASODN was, the greater the inhibition effects were. But concentration-effect saturation appears when drug concentration was higher than 100 μg/ml.

Some references (Augustine, 1997; Bartsch et al., 1998) reported that non-specific effects appeared and side effects increased with the increase dosage. In our study SODN concentration below 100 μg/ml had no inhibition effect. There was a non-specific inhibition effect of ODN, when concentration reached 100 μg/ml. So to find an optimum concentration is essential in order to avoid side effects.

This study showed that the inhibition effect of 100 μg/ml ASODN was greater than that of 50 μg/ml ASODN at 12 hours. The higher the concentration of ASODN, the earlier the peak of inhibition rate, is reached.

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