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In vitro Efficacy of mda-7 Gene for Hepatocellular Carcinoma Gene Therapy Mediated by Human Ribosomal DNA Targeting Vector*

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Abstract Human ribosomal DNA (hrDNA) targeting vector pHr is a homologous recombinant plasmid for human genome which developed in the State Key Laboratory of Medical Genetics. pHr was used to construct a recombinant plasmid pHr-CMG expressing mda-7/GFP fusion gene and its efficacy in the hepatocellular carcinoma cell line Bel-7402 was investigated. The expression of mda-7/GFP fusion gene was detected by fluorescent microscope, RT-PCR and Western blotting, and its function was detected by cell-cycle analyses, MTT assay and Hoechst33258 staining. The results demonstrated that pHr-CMG vector could express MDA-7/GFP fusion protein effectually and the mda-7 gene could induce cell apoptosis and proliferation suppression in Bel-7402 cell line, which might be caused by the G2/M cell cycle arrest. These results also suggested that human ribosomal DNA targeting vector system and the pHr-CMG vector may be applied in further gene therapy researches for hepatocellular carcinoma.

Key words hrDNA targeting vector, mda-7 gene, hepatocellular carcinoma, gene therapy
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Primary hepatocellular carcinoma (HCC) is one of the most lethal malignant tumors in the world. The effect of clinical therapies on HCC is very limited, thus, novel strategies of the treatment for HCC such as gene therapy are considered to be developed.

An important aspect in gene therapy is the selection of the therapeutic gene. mda-7 gene was used in many cancer gene therapy researches. It was identified by subtraction hybridization from a human melanoma cell line [1, 2]. Structural analysis, sequence homology and functional conservation indicated that it belonged to the interleukin IL-10 family of cytokines, therefore, it was redesignated as IL-24[3, 4] and had been localized to chromosome 1q32. mda-7 gene contains seven exons and encodes a 23.8 ku conserved protein with 206 amino acids. Several independent studies using adenovirus expression system had demonstrated that ectopic expression of mda-7 induced growth suppression and apoptosis in a broad spectrum of human cancer, including melanoma, glioblastoma multiforme, mesotheliomas, osteosarcoma and carcinomas of the breast, cervix, colon, lung, nasopharynx, pancreas and prostate, but the ectopic expression has no impact on normal human epithelial, endothelial, smooth muscle or fibroblast cells[5~13]. This unique potentiality of mda-7 gene suggested that it could be applied in cancer gene therapy.

Furthermore, a bottleneck problem in gene therapy researches is the gene transfer system, currently it mainly relies on virus vectors. However, there are many disadvantages like the potential safety concern of viral vectors in the researches, especially

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when clinical trials are involved[14~16]. Since 1994, our laboratory has been working on developing a series of novel non-viral human derived vectors for human gene therapy, which have been extensively applied in the gene therapy researches for single gene inheritance disease and malignant tumor in our laboratory, and we have gained some satisfactory results[17~21]. Among these vectors, pHr is the most efficient one. It could site-directed target exogenous gene into the hrDNA locus of human genome through homologous recombination. This vector was developed for further use in gene therapy research.

In this study, we fused mda-7 gene with GFP gene and combined mda-7/GFP with CMV enhancer/promoter (CMVe/p), then cloned the CMVe/p-mda-7/GFP fragment into pHr, the final recombinant plasmid named as pHr-CMG. pHr-CMG was transferred into human HCC cell line Bel-7402 and its impact on cell proliferation, cell cycle and apoptosis were investigated. The results provided important supports to the use of pHr-CMG in gene therapy for HCC.

1 Materials and methods

1.1 Materials

pHr vector was constructed by our laboratory; pEGFP-N1 plasmid purchased from Clontech; Polymerase, T4 ligase and all restriction enzymes purchased from Takara and MBI; RPMI-1640 medium, MTT reagents and anti-GFP antibody purchased from Sigma; Anti-rabbit-HRP purchased from Abcam; Lipofectamine 2000 and Trizol purchased from Invitrogen; Reverse transcription system purchased from Promega; Enhanced chemiluminescence kit purchased from Amersham; All primers were synthesized by BioAsia Biotechnology; Human HCC cell line Bel-7402 was obtained from China Central For Type Culture Collection.

1.2 Recombinant plasmid construction

Because there is no enhancer or promoter in pHr vector, so it is necessary to add these regulation elements. CMVe/p and mda-7 gene (GenBank No. BC009681 249-872) were amplified by PCR. Primers are shown in Table 1. Amplification products were digested with restriction enzymes, *Bam*H Ⅰ and *Age* Ⅰ for mda-7, *Nhe* Ⅰ and *Xho* Ⅰ for CMVe/p, and then both the fragments were cloned into pEGFP-N1 plasmid. Then the intermediate vector was digested with *Nhe* Ⅰ and *Afl* Ⅱ to generate the CMVe/p-mda-7/GFP fragment, which was cloned into the pHr backbone and the final recombinant plasmid named as pHr-CMG. The expressing cassette was confirmed by sequencing.

1.3 Fluorescent microscope detected expression of mda-7/GFP

Cells were cultured on cytospin slides in a 12-well plate (100 000 cells/well) and transferred with pHr-CMG and pEGFP-N1 vectors at suitable cell density. 24 h later, cytospin slides were fixed in 70% ethanol for 20 min at 4℃. After two washes with PBS, the cells were stained for 10 min with 0.5 g/L DAPI in PBS. The expression of the fusion green fluorescent protein (MDA-7/GFP) was detected by fluorescent microscope.

1.4 RT-PCR

Cells were transfected with pHr-CMG and pHr respectively, the other two groups which treated with Lipofectamine 2000 and RPMI-1640 without serum worked as control. Total RNAs were extracted by Trizol, mRNAs of these four groups were detected by RT-PCR. RNA samples were dealt with Reverse Transcription System and then amplified mda-7 and β-actin genes. Primers were shown in Table 1.

Table 1 The amplification primers(restriction enzyme sites are underlined)

Primer name	Sequence (from 5′ end to 3′ end)	Restriction site	Note
CMVep F	gctagctagttattaatagtaatc	*Nhe* Ⅰ	Primers CMVep F and CMVep R are used to amplify the 601 bp CMVe/p fragment.
CMVep R	ctcgaggatctgacggttcactaa	*Xho* Ⅰ	
Mda-7 F	aggatccgccaccatgaattttcaacagagg	*Bam*H Ⅰ	Primers Mda-7 F and Mda-7 R are used to amplify the 643 bp mda-7 gene with termination codon deletion mutation.
Mda-7 R	gaccggtgagagcttgtagaatttctg	*Age* Ⅰ	
β-Actin F	gctgtccctgtacgcctct		Primers β-Actin F and β-Actin R are used to amplify 360 bp β-actin gene which works as control.
β-Actin R	ctcgttgccgatggtgat		

1.5 Western blotting

24 h after transfection Bel-7402 cells were harvested with 5× loading buffer and incubated for 10 min at 99℃. Cell extracts were separated on 12% SDS-PAGE and electroblotted onto PVDF membrane. Blots were blocked and incubated with anti-GFP antibody and then with anti-rabbit-HRP IgG as a secondary antibody. Hybridization signals were detected using chemiluminescence kit.

1.6 Cell cycle analyses

Cells were cultured in a 6-well plate (～250 000 cells/well) and treated with pHr-CMG, pHr, Lipofectamine 2000 and RPMI-1640 without serum as described above. 48 h later, cells were harvested, washed with PBS and fixed in 70% ethanol overnight at 4℃. Washed with PBS again and approximately 10^6 cells were suspended in 400 μl of PBS containing 10 mg/L of RNase and 5 mg/L of PI. After 30 min of incubation, cells were analyzed by flow cytometry.

1.7 MTT assay for determination of cellular viability

Cells were cultured in a 96-well plate (6 000 cells/well). 24 h later, cells were transfected and treated with pHr-CMG, pHr, Lipofectamine 2000 and RPMI-1640 without serum, respectively. Cell growth was detected *via* MTT assay at 24, 48, 72 h after treatment. Then the plates were analyzed by microplate reader at 570 nm.

1.8 Hoechst 33258 staining test

After 48 h of transfection cells were washed with PBS and fixed in 40 g/L paraformaldehyde for 30 min at room temperature. After washing twice with PBS, cells were stained for 30 min in the dark at room temperature with 0.05 g/L Hoechst 33258 in PBS. Nuclear fragmentation was observed using fluorescence microscope. Apoptotic cells were identified by condensation of nuclear chromatin and fragmentation.

1.9 Statistical analysis

Statistical analysis was performed using one-way ANOVA. Differences with a $P < 0.05$ were considered statistically significant.

2 Results

2.1 Construction of pHr-CMG vector

601 bp CMVe/p fragment and 643 bp mda-7 gene were amplified by PCR (Figure 1a). The final pHr-CMG vector was digested with *Nhe* Ⅰ and *Afl* Ⅱ and generated about 2.3 kb CMVe/p-mda-7/GFP fragment and 8.6 kb pHr backbone fragment (Figure 1b), then the recombinant plasmid pHr-CMG was confirmed by sequencing analysis.

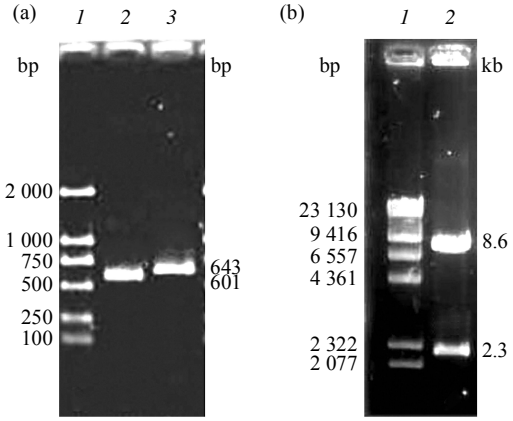

Fig. 1 Identification of PCR fragments and pHr-CMG

(a) Clone of CMVe/p fragment and mda-7 gene by PCR. *1*: DNA marker DL2000; *2*: CMVe/p fragment; *3*: mda-7 gene. (b) Restriction map of the recombinant plasmid pHr-CMG. *1*: *Hin*d Ⅲ marker; *2*: pHr-CMG digested by *Nhe* Ⅰ and *Afl* Ⅱ.

2.2 MDA-7/GFP fusion protein could be expressed in cells

The transfection rate of pHr-CMG vector was approximately 44% through flow cytometry analysis. Expression of MDA-7/GFP fusion protein was located by fluorescent microscope and the representative data were obtained (Figure 2). The cells transfected with pHr-CMG mainly showed granular cytoplasmatic pattern and there was no signal in nucleus, while GFP protein was expressed not only in cytoplasm but also in nucleus.

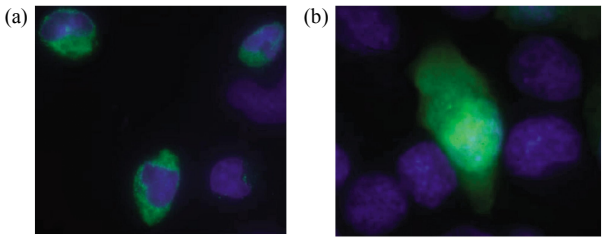

Fig. 2 Location of MDA-7/GFP fusion protein and GFP protein in Bel-7402 cells by fluorescent microscope (40×)

(a) pHr-CMG transfected group. MDA-7/GFP fusion protein was only expressed in cytoplasm. (b) pEGFP-N1 transfected group. GFP protein was expressed both in cytoplasm and nuclear.

Through RT-PCR and Western blotting, MDA-7/GFP fusion protein expressed by pHr-CMG vector was confirmed in cells. RT-PCR showed only

pHr-CMG transferred cells transcribed mda-7 mRNA (Figure 3), meanwhile the result of Western blotting demonstrated that the specific 51 ku MDA-7/GFP fusion protein was detected in this group (Figure 4). These results suggested that even the recombinant plasmid pHr-CMG was 10.9 kb in length, it also could be transferred into cells by Lipofectamine 2000 successfully and express MDA-7/GFP fusion protein effectively with the effect of additional CMVe/p element.

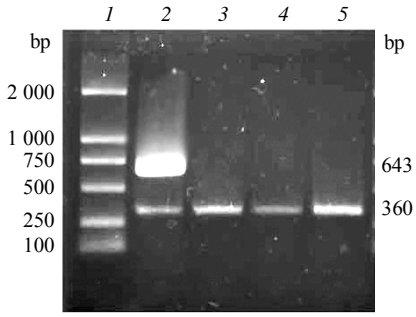

Fig. 3 Identification of mda-7 gene expression by RT-PCR

1: DL2000 DNA marker; *2*: pHr-CMG transfected group. 643 bp mda-7 gene and 360 bp β-actin fragment were obtained; *3*, *4* and *5*: pHr transfected group, Lipofectamine 2000 treated group and untreated group. Only 360 bp β-actin fragment was obtained.

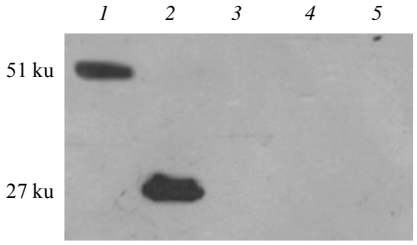

Fig. 4 Identification of MDA-7/GFP fusion protein expression by Western blotting

1: pHr-CMG transfected group. 51 ku specific MDA-7/GFP fusion protein band was obtained; *2*: pEGFP-N1 transfected group. 27 ku GFP control band was obtained; *3*, *4* and *5*: pHr transfected group, Lipofectamine 2000 treated group and untreated group.

2.3 Expression of pHr-CMG suppressed cell proliferation

The effects of pHr-CMG expression in cell cycle were investigated by flow cytometry. As shown in Table 2, after 48 h treatment, the rates of G2/M phase cells were 15.07% in pHr-CMG group, 5.56% in pHr group, 8.31% in Lipofectamine 2000 group and 7.17% in RPMI-1640 without serum treated group. These results indicated that pHr-CMG markedly induced G2/M accumulation in Bel-7402 cell line ($P < 0.05$).

Table 2 Cell cycle distribution of different treatment groups in Bel-7402 cells

Treatment groups	Bel-7402(%)		
	G1	S	G2/M
pHr-CMG	49.17 ± 2.83	35.75 ± 7.00	15.07 ± 4.20
pHr	47.10 ± 6.91	47.34 ± 8.33	5.56 ± 2.02
Lipofectamine 2000	79.04 ± 2.75	12.64 ± 3.73	8.31 ± 2.00
Untreated control	77.59 ± 1.15	15.25 ± 1.78	7.17 ± 1.06

Values are $\bar{x} \pm s$.

In MTT assay experiment, after 24 h, 48 h and 72 h, compared with RPMI-1640 without serum treated control group, the mean cell survivability was 78%, 22% and 13% in pHr-CMG treated group, 93%, 38% and 35% in pHr treated group and 97%, 87% and 81% in Lipofectamine 2000 treated group (Figure 5). These results showed that pHr-CMG significantly inhibited cell proliferation compared with the control group ($P < 0.01$).

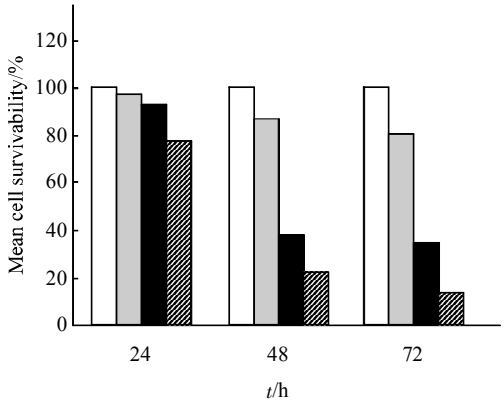

Fig. 5 Mean cell survivability in different treatment cell groups

Cells were treated with Lipofectamine 2000, pHr, pHr-CMG and RPMI-1640 without serum which served as control. Cell survivability was determined *via* MTT proliferation assay 24, 48, 72 h after treatment. Results were expressed as means of samples (n=12) and the pHr-CMG vector could significantly inhibit cell proliferation. □: RPMI-1640 without serum control; ▨: Lipofectamine 2000; ■: pHr; ▨: pHr-CMG.

It was reported that mda-7 gene could increase the rate of G2/M phase cells in melanoma and nonsmall-cell lung cancer *in vitro* [12, 22], it could also suppress cell growth in breast cancer cells [11]. These

effects may be caused by the signaling pathway in which mda-7 gene induces the protein expression of GADD (growth arrest and DNA damage) family, then these proteins, especially the GADD34, leads to growth suppression [6], moreover, mda-7 gene could also activate JNK (c-Jun-NH2-kinase) signaling pathway to inhibit growth[23].

In addition, cell survivability was also decreased in pHr and Lipofectamine 2000 groups mentioned above, these results may be due to the toxicity of Lipid-DNA complex [24], and the survivability of pHr-CMG group was still lower than those observed in pHr and Lipofectamine 2000 groups ($P < 0.01$).

2.4 mda-7 gene mediated apoptosis in hepatocellular carcinoma cells

By Hoechst 33258 staining we observed the apoptosis in different treatment groups. The pHr-CMG group showed low cell density and nuclear chromatin of most cells had condensed, meanwhile the other three groups were normal (Figure 6). The result suggested that MDA-7/GFP could mediate apoptosis remarkably in Bel-7402 cells and the reasons were

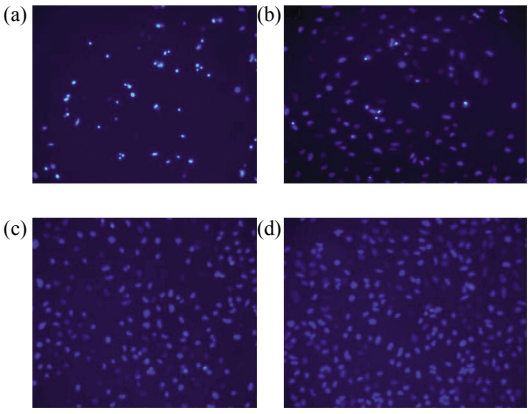

Fig. 6 Apoptosis induced by expression of mda-7 gene in Bel-7402 cells (10×)

(a) pHr-CMG transfected group. The cell density was low and most cells showed nuclear chromatin condensation. (b) pHr transfected group. (c) Lipofectamine 2000 treated group. (d) Untreated group. The last three groups showed very low or no apoptosis.

very complicated. Explanations for this phenomenon may include: 1) Expression of mda-7 gene could change the rate between pro-apoptotic protein and inhibitor of apoptosis proteins, for instance increasing the expression of pro-apoptotic protein like Bax, Fas/Fasl in cells [11, 13, 25]; 2) Up regulate PKR (double-stranded RNA-dependent protein kinase) expression. Expression of mda-7 gene could induce PKR up-regulation quickly and then resulted in apoptosis through phosphorylation of eIF-2α and other substrates [26]; 3) Negatively regulate the β-catenin and PI3K signaling pathways. By this means mda-7 gene could regulate expression of pro-apoptotic protein and oncogene proteins and induced apoptosis [27]. These explanations had been confirmed in some kinds of cancer cell, while not in hepatocellular carcinoma, in which further study has to be carried out for the possible mechanisms. In this study, according to the result of cell-cycle analysis, mda-7 gene may utilize a pathway mediated *via* G2/M cell cycle arrest and then results in apoptosis.

3 Discussion

Expression of mda-7 gene *in vivo* is limited in immunity tissues such as spleen, thymus, peripheral blood leucocyte, monocyte [28]. The mechanism of mda-7 gene action is MDA-7 protein combines with its receptor, a heterodimer, activates the JAK-STAT signaling pathway, and then induces its biological effect especially in immunological regulation [29]. Overexpression of mda-7 gene could affect multiple signal transduction pathways in cancer cells and thus induce growth suppressing and apoptosis, but it displayed a cancer-specific property through both intracellular and secretory pathways [30]. There are few reports about the mda-7 gene in human hepatocellular carcinoma cell. The results in this study indicated that mad-7 gene had the similar effects aforementioned in hepatocellular carcinoma and proposed that the apoptosis in Bel-7402 cells is mainly caused by the G2/M cell cycle arrest. These results provided strong evidences for hepatocellular carcinoma gene therapy using mda-7 gene and also supported the use of pHr vector in gene therapy researches.

As a novel non-viral vector system derived from human chromosomes, pHr can target exogenous gene into hrDNA locus and shows potencial advantages in gene therapy studies. Compared with viral vectors, pHr has its unique characteristics: 1) It targets the exogenous therapeutic genes to hrDNA locus by homologous recombination, which makes the therapeutic genes express stably; 2) pHr could express exogenous gene effectively because of the high transcription efficiency of genes at the ribosomal DNA locus; 3) pHr originated from human chromosomes,

thus it is non-immunogenic. Therefore，we think pHr is a good potential vector for gene therapy.

mda-7 gene has other properties such as inhibiting neovascularization in cancer, increasing the tumor radiosensitivity, so it is an efficient anti-cancer gene. Combined with pHr human targeting vector system, the pHr-CMG vector may show more advantages in further researches in hepatocellular carcinoma gene therapy.

References

1 Jiang H, Lin J J, Su Z Z, *et al*. Subtraction hybridizaion identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression. Oncogene, 1995, **11**(12): 2477～2486

2 Jiang H, Lin J J, Su Z Z, *et al*. The melanoma differentiation associated gene mda-7 suppresses cancer cell growth. Proc Natl Acad Sci USA, 1996, **93** (17): 9160～9165

3 Chada S, Sutton R B, Ekmekcioglu S, *et al*. MDA-7/IL-24 is a unique cytokine-tumor suppressor in the IL-10 family. Int Immunopharmacol, 2004, **4**(5): 649～667

4 Pestka S, Krause C D, Sarkar D, *et al*. Interleukin-10 and related cytokines and receptors. Annu Rev Immunol, 2004, **22**: 929～979

5 Allen M, Pratscher B, Roka F, *et al*. Loss of nover mda-7 splice variant (mda-7s) expression is associated with metastatic melanoma. J Invest Dermatol, 2004, **123**(3): 583～588

6 Sarkar D, Su Z Z. Lebedva I V, *et al*. mda-7 (IL-24) mediates selective apoptosis in human melanoma cells by inducing the coordinated overexpression of the GADD family of genes by means of p38 MAPK. Proc Natl Acad Sci USA, 2002, **99**(15): 10054～10059

7 Su Z Z, Lebedva I V, Sarkar D, *et al*. Melanoma differentiation associated gene-7, mda-7/IL-24, selectively induced growth suppression, apoptosis and radiosensitization in malignant gliomas in a p53-independent manner. Oncogene, 2003, **22**(8): 1164～1180

8 Gopalkrishnan R V, Sauane M, Fisher P B. Cytokine and tumor cell apoptosis inducing activity of mda-7/IL-24. Int Immunopharmacol, 2004, **4**(5): 635～647

9 Madireddi M T, Su Z Z, Young C S, *et al*. mda-7, a novel melanoma differentiation associated gene with promise for cancer gene therapy. Adv Exp Med Biol, 2000, **465**: 239～261

10 Jiang H, Su Z Z, Lin J J, *et al*. The melanoma differentiation associated gene mda-7 suppresses cancer cell growth. Proc Natl Acad Sci USA, 1996, **93**(17): 9160～9165

11 Su Z Z, Madireddi M T, Lin J J, *et al*. The cancer growth suppressor gene mda-7 selectively induces apoptosis in human breast cancer cells and inhibits tumor growth in nude mice. Proc Natl Acad Sci USA, 1998, **95**(24): 14400～14405

12 Saeki T, Mhashilkar A, Chada S, *et al*. Tumor-suppressive effects by adenovirus-mediated mda-7 gene transfer in non-small cell lung cancer cell *in vitro*. Gene Ther, 2000, **7**(23): 2051～2057

13 Su Z, Lebedeva I V, Gopalkrishnan R V, *et al*. A combinatorial approach for selectively inducing programmed cell death in human pancreatic cancer cells. Proc Natl Acad Sci USA, 2001, **98** (18): 10332～10337

14 Armentano D, Yu S F, Kantoff P W, *et al*. Effect of internal viral sequence on the utility of retroviral vectors. J. Virol, 1987, **61** (5): 1647～1650

15 Anson D S. The use of retroviral vectors for gene therapy-what are the risks? A review of retroviral pathogenesis and its relevance to retroviral vector-mediated gene delivery. Genet Vaccines Ther, 2004, **2**(1): 9

16 Jozkowicz A, Dulak J. Helper-dependent adenoviral vectors in experimental gene therapy. Acta Biochim Pol, 2005, **52**(3): 589～599

17 Liu X H, Liu M J, She H, *et al*. Expression of reconstructive hFⅧ in the hrDNA by using hrDNA targeting vector. Chinese Science Bulletin, 2005, **50**(18): 1972～1977

18 Liang Y, Liang D S, Xue Z G, *et al*. A minidystrophin-EGFP fusion gene expressed in Cos-7 cells mediated by human source vector. Chin J Med Genet, 2005, **22**(5): 493～496

19 Xia K, Liang D S, Tang A F, *et al*. A novel fusion suicide gene yeast CDglyTK plays a role in radio-gene therapy of nasopharyngeal carcinoma. Cancer Gene Therapy, 2004, **11**(12): 790～796

20 Wang L N, Xue Z G, Li Z, *et al*. Investigation of hrDNA targeting vector-mediated tumor-specific suicide gene therapy for hepatocellular carcinoma. Chinese Science Bulletin, 2006, **51** (19): 2342～2350

21 Liu X H，Liu M J，Xue Z G, *et al*. Non-viral *ex vivo* transduction of human hepatocyte cells to express factor VIII using a human ribosomal DNA-targeting vector. Thromb Haemost, 2007, **5** (2): 347～351

22 Lebedeva I V, Su Z Z, Chang Y, *et al*. The cancer growth suppressing gene mda-7 induces apoptosis selectively in human melanoma cells. Oncogene, 2002, **21**(5): 708～718

23 Yacoub A, Mitchell C, Lebedeva I V, *et al*. mda-7 (IL-24) inhibits growth and enhances radiosensitivity of glioma cells *in vitro via* JNK signaling. Cancer Biol Ther, 2003, **2**(4): 347～353

24 Felgner P L, Gadek T R, Holm M, *et al*. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc Natl Acad Sci USA, 1987, **84**(21): 7413～7417

25 Gopalan B, Litvak A, Sharma S, *et al*. Activation of the Fas-FasL signaling pathway by MDA-7/IL-24 kills human ovarian cancer cells. Cancer Res, 2005, **65**(8): 3017～3024

26 Paster A, Vorburger S A, Barber G N, *et al*. Adenoviral transfer of the melanoma differentiation-associated gene 7 (mda-7) induces apoptosis of lung cancer cells *via* up-regulation of the double-stranded RNA-dependent protein kinase(PKR). Cancer Res, 2002, **62**(8): 2239～2243

27 Mhashilkar A M, Stewart A L,Sieger K, *et al*. MDA-7 negatively regulates the beta-catenin and PI3K signaling pathways in breast and lung tumor cells. Mol Ther, 2003, **8**(2): 207～219

28 Huang E Y, Madireddi M T, Gopalkrishnan R V, *et al*. Genomic

structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (mda-7) gene with cancer specific growth suppressing and apoptosis inducing properties. Oncogene, 2001, **20**(48):7051～7063

29 Mhashilkar A M, Schrock R D, Hindi M, *et al*. Melanoma differentiation associated gene-7 (mda-7): a novel anti-tumor gene for cancer gene therapy. Mol Med, 2001, **7**(4): 271～282

30 Sauane M, Lebedeva I V, Su Z Z, *et al*. Melanoma differentiation associated gene-7/Interleukin-24 promotes tumor cells-specific apoptosis through both secretory and nonsecretory pathways. Cancer Res, 2004, **64**(9): 2988～2993

人核糖体 DNA 打靶载体介导 mda-7 基因对肝癌的体外基因治疗研究 *

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摘要 人核糖体 DNA 打靶载体 pHr 是一种由中南大学医学遗传学国家重点实验室开发构建的针对人类基因组的同源重组质粒载体．利用 pHr 构建了一种 mda-7/GFP 融合基因的人源基因表达载体 pHr-CMG，并研究了其在肝癌细胞系 Bel-7402 中的作用．利用荧光显微镜、RT-PCR 和 Western blotting 检测了 mda-7/GFP 融合基因的表达；利用细胞周期分析、MTT 和 Hoechst33258 染色研究了其在细胞中的作用．结果显示，pHr-CMG 载体能在 Bel-7402 细胞中有效表达 MDA-7/GFP 融合蛋白，进而抑制细胞增殖和诱导细胞凋亡，推测其可能是由载体表达了 mda-7 基因引起细胞在 G2/M 期累积所导致的．同时，实验结果证实了人核糖体 DNA 打靶载体系统以及 pHr-CMG 表达载体的有效性，为其在进一步基因治疗研究中的应用提供了理论和实验基础.

关键词 人核糖体 DNA 打靶载体，mda-7 基因，肝癌，基因治疗
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