PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2011, 38(8): 737~743
www.pibb.ac.cn

Lentivirus-mediated RNA Interference of ppGalNAc-T2 Gene Expression Inhibit Proliferation and Migration of Jurkat Cell Line*

LIU Chun-Liang[1)], LIN Dan-Dan[2)], XU Lan[1)], JIANG Zhi[1)], WU Shi-Liang[1)**]
([1)] *Department of Biochemistry and Molecular Biology, Medical College, Institute of Bioengineering, Soochow University, Suzhou* 215123, China;
[2)] *Department of Immunology, Soochow University, Suzhou* 215123, China)

Abstract Mucin O-glycosylation plays important roles in many carcinogenic events, and it is initiated by the enzymes: UDP-GalNAc：polypeptide N-acetylgalactosaminyltransferases (ppGalNAc-Ts). The association of ppGalNAc-T2 expression and Jurkat cells proliferation and migration was investigated. The RNAi and negative control shRNA were synthesized and then inserted into lentivirus vector YH1. After enzyme digestion and sequencing confirmation, each of the recombinant vectors and packaging vectors were cotransducted into 293T cells, and the recombinant lentivirus were packaged. Then Jurkat cells were infected by purified lentivirus, RT-PCR and Western blot showed that ppGalNAc-T2 mRNA and protein expression were remarkably decreased after RNA interference. Furthermore, the proliferation and migration abilities of Jurkat cells were detected. In conclusion, the recombinant lentivirus interfering vector was constructed targeting on ppGalNAc-T2 gene, and decreased expression of ppGalNAc-T2 gene inhibit proliferation and migration of Jurkat cells.

Key words ppGalNAc-T2, lentivirus, RNA interference, Jurkat, proliferation, migration
DOI: 10.3724/SP.J.1206.2011.00018

Cell surface-bound and secreted mucins from epithelial and other mucin-producing cells contain O-glycans that comprise approximately 55% of the mucin molecule by weight. The expression of mucins is often altered in cancer, with frequent aberrant glycosylation, resulting in the formation of tumor-associated glycans[1-2]. It has been reported that the tumor-associated glycans play important roles in many carcinogenic events, such as cellular growth, differentiation, transformation, adhesion, invasion and immune surveillance[1-5]. Changes in mucin O-glycans are determined by the expression of the enzymes that initiate O-glycosylation: UDP-GalNAc：polypeptide N-acetylgalactosaminyltransferases (ppGalNAc-Ts). This initial key step controlling mucin O-glycosylation is performed by the family of ppGalNAc-Ts that catalyze the transfer of GalNAc from UDP-GalNAc to serine and threonine residues on the protein synthesizing the Tn antigen[6-7]. To date, over fifteen distinct members of human ppGalNAc-T family have been identified[6-8].

Altered expression of ppGalNAc-Ts could be one of the mechanisms that explain the changes in mucin O-glycosylation during malignant transformation [9]. Variations of the ppGalNAc-Ts expression pattern have been described in many human tumors, for example, decreased expression of ppGalNAc-T1 and increased expression of ppGalNAc-T2 and ppGalNAc-T3 have been reported in oral squamous cell carcinoma, compared with the expression pattern in normal oral mucosa. Compared with normal colonic epithelium, higher expression of ppGalNAc-T1,

*This work was supported by grants from The National Natural Science Foundation of China (30670462).
**Corresponding author.
Tel: 86-512-65880407, E-mail: wushiliang@suda.edu.cn
Received: January 10, 2011 Accepted: March 22, 2011

ppGalNAc-T2, and ppGalNAc-T3 has also been reported in colorectal carcinoma. Different expression patterns of ppGalNAc-T3 have been described in patients with colorectal, lung, pancreatic, gastric, gallbladder, prostate, extrahepatic bile duct and breast carcinomas. T6 was also described in association with breast and gastric carcinoma [10]. ppGalNAc-T2 gene was up-regulated in breast tumors compared to control samples[11], and our laboratory has demonstrated that its expression was also changed during 1,25 $(OH)_2D_3$ induced differentiation in several leukemia cell lines[12]. Based on these studies ppGalNAc-T2 could be considered an interesting marker for the glycosylation modifications in malignancy with implication in molecular diagnosis. Here we construct the recombinant lentivirus, and show that interference of ppGalNAc-T2 expression by lentivirus-mediated RNAi inhibits cell proliferation and migration of Jurkat cells.

1 Materials and methods

1.1 Materials

Jurkat and MEG-01 cells were generously provided by The Insitute of Hematology of Soochow University, and cultured in RPMI-1640 (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS). To generate lentiviruses, 293T cells were used, and grown in DMEM containing 10% FBS.

1.2 Lentivirus vectors construction

The positive siRNA sequence(5′ TGGCAGGTT-TACGTCAATA 3′) and the negative control sequence (5′ ATAGGGCGTACGATTATCT 3′) to ppGalNAc-T2 gene have been designed[13], then we added a loop ring (ttcaagaga) for shRNA. The ppGalNAc-T2 hairpin oligo (5′ GATCCCCTGGCAGGTTTACGTCAATA-ttcaagagaTATTGA CGTAAACCTGCCATTTTTGGA-AA 3′, 5′ AGCTTTTCCAAAAATGGCAGGTTT-ACGTCAATAtctcttgaaTATTGACGTAAACCTGCC-AGGG 3′) were synthesized (Sangon Biotech, Shanghai) and annealed. The annealed double stranded shRNA oligo was cloned into pRRL-YH1-YFP Amp lentivirus vector (A gift from Dr. Baum C, Hannover Medical College) that carrying the transgene for yellow fluorescent protein (YFP). A control shRNA unrelated to human gene sequences was used as a negative control (5′ GATCCCCATAGGGCGTACG-ATTATCTttcaagagaAGATAATCGTACGCCCTATT-TTTTGGAAA 3′, 5′ AGCTTTTCCAAAAAATAGG-GCGTACGATTATCTtctcttgaaAGATAATCGTACG-CCCTATGGG 3′). The recombinant vectors were identified using endonuclease by *Bam*H Ⅰ and *Nhe* Ⅰ and then confirmatory sequencing.

1.3 Lentivirus generation

The recombinant ppGalNAc-T2 shRNA lentiviruses were produced by transient transfection of 293T cells according to standard protocol[14]. Briefly, subconfluent 293T cells on 10 cm plates were cotransfected with 10 μg of the vector, 6.5 μg ΔR, 3.5 μg VSV-G and 2.5 μg Rev by calcium phosphate precipitation. After 24 h medium was changed, and lentiviral particles were obtained after 48 h, The YFP expression in 293T was also identified.

1.4 Jurkat transduction and selection

For transduction, Jurkat cells were plated into 3.5 cm dish and allowed to adhere for 16 h. Cells were infected with either ppGAlNAc-T2 siRNA lentivirus or negative control in complete medium for 72 h. Cells were washed and switched into complete medium for 72 h. Then the positive cells was sorted into 96-well plate (1 cell/well) by High-speed cell sorter (Beckman Coulter) after 72 h. Three weeks later, the > 99% YFP positive cells were selected to construct the stable transfected cell lines. Then cells were harvested for infected proportion analysis or cell proliferation and apoptosis assay, and so on.

1.5 Semi-quantitative PCR

Total RNA was prepared from the transfected cells with Trizol reagent. cDNA was synthesized with reverse transcriptase and oligo dT primers. The following primer pairs were used: ppGAlNAc-T2 (669 bp), forward 5′ AAGAAAGACCTTCATCAC-AGCAATGGAGAA, reverse 5′ ATCAAAACCGC-CCTTCAAGTCAGCA; β-MG (330 bp), forward 5′ CTCGTGCTACTCTCTCTTTC, reverse 5′ CAT-GTCTCGATCCCACTTAAC. β-MG was used as an internal control. PCR was performed for 25 cycles (94℃ 5 min, then 94℃ 45 s, 56℃ 45 s, 72℃ 45 s, then 72℃ 7 min). PCR products were electrophoresed in 1% agarose gels. Spectrophotometry was used to quantify cDNA levels.

1.6 Western blotting

ppGAlNAc-T2 protein was separated on 12% SDS-PAGE gel and transferred onto PVDF membrane (Millipore) under constant current of 110 mA, 2 h. The PVDF membrane was incubated with rabbit anti-ppGAlNAc-T2 polyclonal antibody (prepared by our laboratory), followed by secondary Abs goat anti rabbit IgG conjugated with HRP (BioWorld), and

developed by ECL-advanced Western detection kit (Beyotime).

1.7 MTT assay

Cell survival was determined using the 3- (4,5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) (Sigma, USA). Counted the number of the cells and added 200 μl (5000 cells) into each well of 96-well plate and incubated for 24 h, 48 h and 72 h. Added 20 μl of 5 g/L MTT to each well, followed by 4 h of incubation. Medium was removed from the wells and 150 μl dimethyl sulfoxide (DMSO) was added to each well, to dissolve formazan crystals. A_{490} was measured by microplate reader (Biotek).

1.8 Transwell assay

Cell migration was assessed by Transwell (Costar, Corning Life Science USA, 5 μm). 1×10^5 cells in 150 μl serum-free RPMI-1640 that placed onto the upper chamber and 600 μl 10% FBS RPMI-1640 in the lower chamber were incubated for 12 h. Cells that had migrated through the filter pores into the lower chamber were collected and detected the number of the cells by MTT assay. Each experiment was done in triplicate and $\bar{x} \pm s$ are presented.

1.9 Statistical analysis

Statistical analysis was performed using SPSS17.0 software. The two-tailed Student t test was used to determine any statistically significant difference. $P < 0.05$ was considered statistically significant.

2 Results

2.1 Identification of lentivirus vectors

The recombinant vectors were identified using endonuclease by *Bam*H Ⅰ and *Nhe* Ⅰ. As shown in Figure 1, the small fragment of YH1 vector(lane *1*) is 254 bp, and YH1 ppGalNAc-T2 shRNA (lane *2*) and YH1 negative control are 318 bp. As we known, the length of shRNA is 64 bp (318 bp –254 bp), after confirmatory sequencing we get the recombinant vectors.

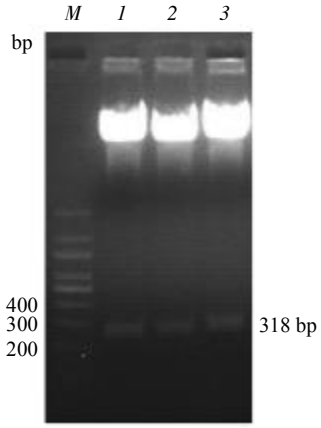

Fig. 1 Confirmation of recombinant plasmids through enzyme digestion

All plasmid were digested by *Bam*H Ⅰ and *Nhe* Ⅰ, *M*: DNA100-bp marker; the small fragment of lane*1* is 254 bp, both of lane *2* and lane *3* are 318 bp.

2.2 Production of recombinant lentivirus

The vectors were cotransfected into 293T cells, after 24 h medium was changed, and lentiviral particles were obtained after 48 h. The YFP expression in 293T was identified by fluorescence microscope (Figure 2), and the infection efficiency was very high.

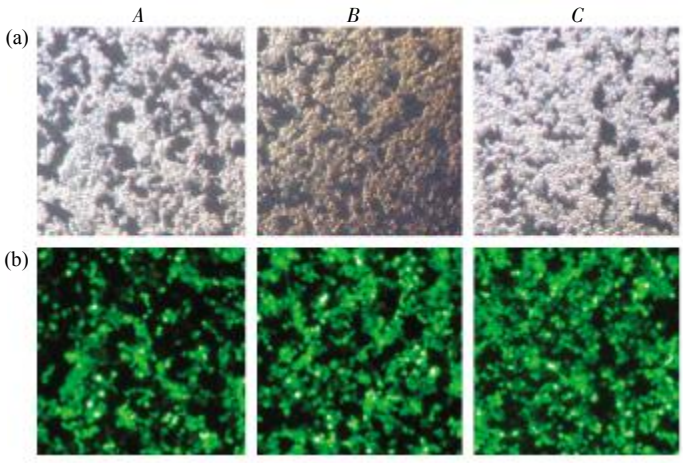

Fig. 2 Production of lentivirus in 293T cells

The vectors were transducted into 293T cells, 72 h later, the supernatant was collected, and infection efficiency were determined according to the expression of the reporter gene *YFP*. (a) Cultured 293T cells before transfection. (b) The YFP expression of 293T cells after transfection with YH1(*A*), YH1 ppGalNAc-T2 shRNA (*B*) and YH1 negative control(*C*). 40×.

2.3 The proportion of transducted Jurkat cells using FACS

To inhibiting ppGalNAc-T2 expression in Jurkat cells, we used the shRNA lentivirus vector to express ppGalNAc-T2 hairpin siRNA in transducted cell. Jurkat were infected with either the YH1 vector, hairpin siRNA vector or the negative control siRNA vector. After the transducted Jurkat cells were selected by High-speed cell sorter, we analyzed the proportion of transducted Jurkat cells using FACS analysis. The transducted Jurkat cells expressed YFP proteins after infected with lentivirus vectors. Results of FACS analysis confirmed that over 99% Jurkat cells were transducted with YH1 vector (Figure 1a), lentivirus shRNA vector(Figure 1b) and Control vector(Figure 1c). Our data suggested that lentivirus vector had high efficiency for infecting Jurkat cells.

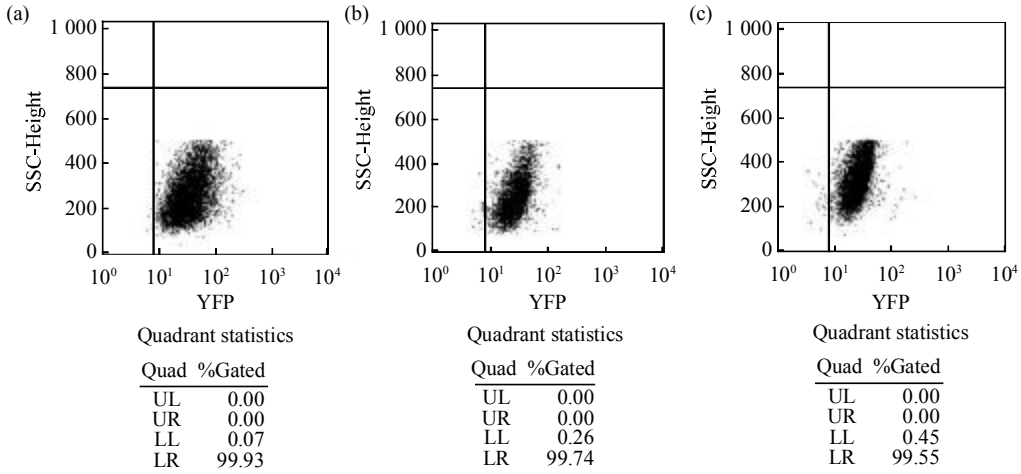

Fig. 3 Expression of YFP by flow cytometry analysis

The Jurkat cells were transducted with lentivirus vector expressed YFP. Cells transducted with lentivirus plasmids were analyzed with FACS. The proportion of Jurkat cells transducted with YH1(a), YH1 ppGalNAc-T2 shRNA (b) and YH1 negative control(c) were all over 99%.

2.4 Lentivirus-mediated RNAi inhibits ppGalNAc-T2 mRNA and protein expression

Semi-quantitative PCR analysis demonstrated that endogenous ppGalNAc-T2 mRNA of Jurkat cells transfected with ppGalNAc-T2 hairpin siRNA vector markedly decreased compared to the YH1 vector, the negative control siRNA vector transfected cells and untransfected cells (Figure 4a). We also examined the effect of ppGalNAc-T2 siRNA on the levels of ppGalNAc-T2 protein by Western blot analysis. Figure 4b showed that RNAi targeting ppGalNAc-T2 significantly inhibited the expression of ppGalNAc-T2 protein in Jurkat cells compared to cells transfected with control siRNA vector and untransfected cells.

2.5 Analysis of cell proliferation

To determine whether silencing ppGalNAc-T2 by RNAi had an inhibitory effect on Jurkat cells proliferation, we accomplished determination of cell proliferation with MTT assay. Figure 5 showed that inhibition of ppGalNAc-T2 expression by RNAi suppressed proliferation of Jurkat cells.

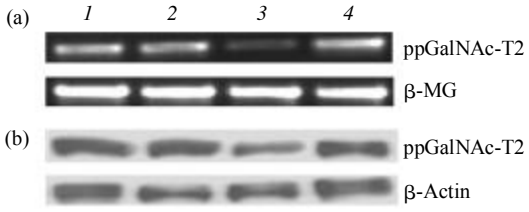

Fig. 4 Detection of ppGalNAc-T2 mRNA and protein expression

(a) Total RNA was isolated from Jurkat (*1*) cells and Jurkat cells transfected with YH1 (*2*), ppGalNAc-T2 siRNA expression vector (*3*) or control siRNA vector (*4*). Semi-quantitative PCR was performed to measure the relative copies of ppGalNAc-T2 mRNA. The amount of each product was normalized to the housekeeping gene β-MG. ppGalNAc-T2 mRNA in Jurkat cells decreased significantly after infected with lentivirus shRNA vector targeting ppGalNAc-T2 gene. (b) Western blot analyzed expression and activation of ppGalNAc-T2 proteins in Jurkat cells transfected with YH1 vector (*2*), ppGalNAc-T2 siRNA expression vector (*3*) or control siRNA vector (*4*). Cell lysates were subjected to electrophoresis on an SDS-PAGE gel and processed for Western blotting with anti- ppGalNAc-T2 or anti-β-actin antibodies. Equal amount of protein was loaded on each gel lane. ppGalNAc-T2 siRNA inhibited significantly expression of ppGalNAc-T2 in Jurkat cells.

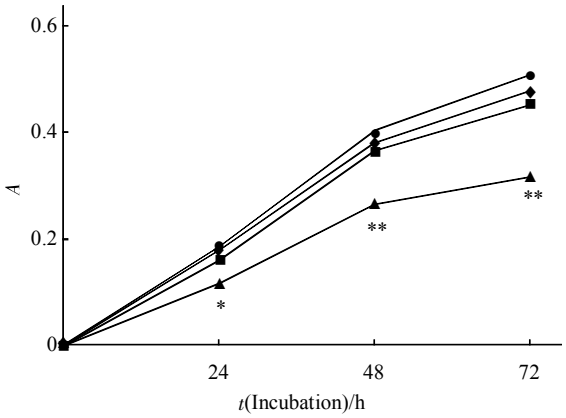

Fig. 5 Cell proliferation evaluated by MTT assays

The protracted cell growth curve and the results of the inhibitory rates of cell growth were calculated based on absorbance at 490 nm. Each value represents the mean of five replicates. The proliferation of Jurkat cells transfected with YH1-shRNA was suppressed significantly compared with cells without transfection or transfected with YH1 or YH1 control. ●—●: Jurkat; ■—■: YH1; ♦—♦: YH1-control; ▲—▲: YH1-shRNA.

2.6 Analysis of cell migration

To test whether ppGalNAc-T2 expression affects motility of Jurkat cell line, we carried out standard *in vitro* chamber assays. As shown in Figure 6, the *A* value of Jurkat cells transfected with ppGalNAc-T2 siRNA vector (*3*) was significantly lower than others. This result suggested that migration of Jurkat cells transfected with ppGalNAc-T2 siRNA vector (*3*) was reducing compared with Jurkat cells (*1*), transfected with YH1(*2*) or control siRNA vector (*4*).

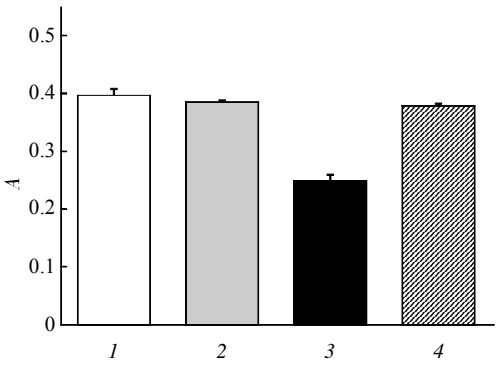

Fig. 6 Reducing migration abilities of Jurkat cells by lentivirus-mediated RNAi

The number of the migrated cells was detected by MTT assay. Each value represents the mean of five replicates. The migration activity of Jurkat shRNA cells (*3*) was reduced compared with Jurkat cells (*1*), transfected with YH1(*2*) or control siRNA vector(*4*).

3 Discussion

One of the most notable differences between cancer and normal cells is the mucin-type O-glycans. The aberrant O-glycans attached to mucin core proteins have been reported as tumor-associated antigens in many human cancers, including Tn and T antigens. It could be hypothesized that these carbohydrate antigens are the results of the changed expression level of ppGalNAc-T and/or changed expression of other glycosyltransferases that catalyze further steps in the elongation of the O-glycans. Although this is an important question in cancer research, the answer is still controversial. Our study aimed to address this question, for the first time, successfully suppressed the expression of ppGalNAc-T2 using lentivirus-mediate RNAi in leukemia cell line, and found that decreased ppGalNAc-T2 expression inhibit the proliferation and migration abilities of Jurkat cells.

There are so many members in this family, and some of them express widely in many tissues and cells, especially ppGalNAc-T2[15]. Some research have demonstrated that aberrant expression of ppGalNAc-T2 were found in colorectal carcinoma and breast cancer tissue[11], when we study the expression levels of ppGalNAc-Ts in some leukemia cell lines during cell differentiation induced by 1, 25 $(OH)_2D_3$, we found obviously increased expression of ppGalNAc-T2[12]. So we speculate that the expression of ppGalNAc-T2 in leukemia cells have important roles. Recent study found that leukemia Jurkat cells generally showed simple glycan profiles and commonly contained sialyl-T (NeuAcα2-3Galβ1-3GalNAc) and disialyl-T (NeuAcα2-3Galβ1-3 (NeuAcα2-6)GalNAc) antigens as major O-glycans[16]. When the expression of ppGalNAc-T2 changed, the simple glycan antigens will changed.

In order to research the function of ppGalNAc-T2 expression in Jurkat cells, we choose the RNA interference that delivered by Lentivirus. The siRNA can be used effectively *in vitro*/*in vivo* to suppress target-gene expression in eukaryotic cells[17]. But the effect of siRNAs that suppressed the targeting gene expression only keeps a few days. Lentivirus, is a new tool for gene therapy [18-20], could suppress gene long-term expression. The results of FACS demonstrated that the YFP positive rate of lentivirus

vectors transducting to Jurkat cells were over 99%. The RT-PCR and Western blot analysis indicated that the expression of ppGalNAc-T2 in Jurkat cells transducted with ppGalNAc-T2 shRNA vector was significantly suppressed. In the present study, our data reveal that ppGalNAc-T2 siRNA suppressed ppGalNAc-T2 expression in Jurkat cells. Lentivirus vector can stably integrate ppGalNAc-T2 into the chromosomes of their targeting cells, a likely requisite for long-term expression. Therefore, lentivirus shRNA vector is a useful tool with highly efficiency to inhibiting target-gene expression in leukemia cells.

Then we analyzed the Jurkat cells proliferation and migration abilities after transducted by ppGalNAc-T2 siRNA, and found that suppressed expression of ppGalNAc-T2 inhibited Jurkat cells proliferation and migration. The expression of ppGalNAc-T2 is decreased, the synthesis of O-glycan will be changed. C2GnT-1 is another glycosyltransferase catalyzing O-glycan chains, after BCP-leukemia cells was transfected by antisense C2GnT-1 cDNA the C2GnT-1 expression was downregulated, cell adhesion and migration assay demonstrated that the leukemia cells ability of tissue infiltration was obviously inhibited [21]. When the O-GalNAc-based inhibitors were used for colorectal cell research, they found that colorectal cells growth was inhibited through induction of apoptosis and inhibition of proliferation gene expression [22]. In this study, we inhibited the synthesis of O-glycans, that is important for Jurkat cell proliferation and migration.

In conclusion, our investigation demonstrated that lentivirus-mediated RNA interference targeting ppGalNAc-T2 might reduce the proliferation and migration of Jurkat cells. ppGalNAc-T2 and mucin O-glycans have considerable potential as a new therapeutic target for the treatment of leukemia.

References

[1] Hollingsworth M A, Swanson B J. Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer, 2004, **4**(1): 45-60

[2] Springer G F. T and Tn, general carcinoma autoantigens. Science, 1984, **224**(4654): 1198-206

[3] Dube D H, Bertozzi C R. Glycans in cancer and inflammation-potential for therapeutics and diagnostics. Nat Rev Drug Discovery, 2005, **4**(6): 477-488

[4] Brockhausen I. Pathways of O-glycan biosynthesis in cancer cells. Biochim Biophys Acta, 1999, **1473**(1): 67-95

[5] Marth J D, Grewal P K. Mammalian glycosylation in immunity. Nat Rev Immunol, 2008, **8**(11): 874-887

[6] Ten Hagen K G, Fritz T A, Tabak L A. All in the family: the UDP-GalNAc： polypeptide N-acetylgalactosaminyltransferases. Glycobiology, 2003, **13**(1): 1R-16R

[7] Brockhausen I. Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. EMBO Reports, 2006, **7**(6): 599-604

[8] Peng C, Togayachi A, Kwon Y D, *et al*. Identification of a novel human UDP-GalNAc transferase with unique catalytic activity and expression profile. Biochem Biophys Res Comm, 2010, **402** (4): 680-686

[9] Hanisch F G, Reis C A, Clausen H, *et al*. Evidence for glycosylation-dependent activities of polypeptide N-acetylgalactosaminyltransferases rGalNAc-T2 and -T4 on mucin glycopeptides. Glycobiology, 2001, **11**(9): 731-740

[10] 刘春亮, 吴士良. 黏蛋白型 O- 聚糖在人类肿瘤中的结构异常及生物学功能. 生物化学与生物物理进展, 2010, **37**(5): 475-483
Liu C L, Wu S L. Prog Biochem Biophys, 2010, **37**(5): 475-483

[11] Potapenko I O, Haakensen V D, Ludersc T, *et al*. Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Molecular Oncology, 2010, **4**(2): 98-118

[12] Qiu H, Guo X H, Mo J H, *et al*. Expressions of polypeptide: N-acetylgalactosaminyltransferase in leukemia cell lines during 1, 25-dihydroxyvitamin D3 induced differentiation. Glycoconj J, 2006, **23**(7-8): 575-584

[13] 仇 灏, 刘可人, 朱俐燕, 等. 人多肽: N- 乙酰氨基半乳糖转移酶 2 (ppGalNAc-T2) RNA 干扰载体的构建及鉴定. 江苏大学学报(医学版), 2007, **17**(2): 102
Qiu H, Liu K R, Zhu L Y, *et al*. J Jiangsu University (Medicine Edition), 2007, **17**(2): 102

[14] Zufferey R, Nagy D, Mandel R J, *et al*. Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. Nat Biotechnol, 1997, **15**(9): 871-875

[15] Bennett E P, Hassan H, Clausen H. cDNA cloning and expression of a novel human UDP-N-acetyl-α-D-galactosamine Polypeptide-N-Acetylgalactosaminyltransferase, GalNAc-T3. J Biol Chem, 1996, **271**(29): 17006-17012

[16] Yamada K, Kinoshita M, Hayakawa T, *et al*. Comparative studies on the structural features of O-glycans between leukemia and epithelial cell lines. J Proteome Research, 2009, **8**(2): 521-537

[17] Hannon G J. RNA interference. Nature, 2002, **418**(6894): 244-251

[18] Naldini L, Blömer U, Gallay P, *et al*. *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science, 1996, **272**(5259): 263-267

[19] 杨印祥, 李艳华, 刘庆斌, 等. NRSF 慢病毒干涉载体的构建及功能初步检测. 生物化学与生物物理进展, 2008, **35**(2): 151-158
Yang Y X, Li Y H, Liu Q B, *et al*. Prog Biochem Biophys, 2008, **35**(2): 151-158

[20] Herold M J, van den Brandt J, Seibler J, *et al*. Inducible and reversible gene silencing by stable integration of an shRNA-encoding lentivirus in transgenic rats. Proc Natl Acad Sci

USA, 2008, **105**(47): 18507−18512

[21] Kikuchi J, Ozaki H, Nonomura C, *et al*. Transfection of antisense core 2 β1,6-N-acetylglucosaminyltransferase-1 cDNA suppresses selectin ligand expression and tissue infiltration of B-cell precursor leukemia cells. Leukemia, 2005, **19**(11): 1934−1940

[22] Patsos G, Hebbe-Viton V, Robbe-Masselot C, *et al*. O-Glycan inhibitors generate aryl-glycans, induce apoptosis and lead to growth inhibition in colorectal cancer cell lines. Glycobiology, 2009, **19**(4): 382−398

慢病毒介导的 RNA 干扰 ppGalNAc-T2 基因表达抑制 Jurkat 细胞增殖和迁移 *

刘春亮[1)]　林丹丹[2)]　徐　岚[1)]　姜　智[1)]　吴士良[1)**]

([1)] 苏州大学医学部生物化学与分子生物学系暨生化工程研究所，苏州 215123；[2)] 苏州大学免疫学系，苏州 215123)

摘要　在肿瘤中，黏蛋白 O- 糖基化有着重要的生物学功能．控制 O- 糖基化起始合成的是多肽：N- 乙酰氨基半乳糖转移酶家族，研究该酶家族对阐明 O- 糖基化在肿瘤中的作用机制有重要的意义．探讨了靶向干扰 ppGalNAc-T2 基因表达对白血病 Jurkat 细胞株增殖及迁移的影响．首先合成 ppGalNAc-T2 特异 shRNA 干扰及对照序列，将其连接至慢病毒干扰载体 YH1；重组载体经双酶切、测序鉴定正确后与包装质粒共转染 293T 细胞，获得的病毒颗粒经过滤纯化后感染 Jurkat 细胞，流式细胞分选仪进行细胞分选以获得 ppGalNAc-T2 基因稳定干扰表达的 Jurkat 细胞，然后使用 RT-PCR 和 Western blot 方法对各组别细胞中 ppGalNAc-T2 基因表达情况进行分析，以确定 ppGalNAc-T2 基因表达被有效干扰；进一步利用 MTT 实验和 Transwell 实验分析 ppGalNAc-T2 基因干扰表达对 Jurkat 细胞增殖及迁移的影响．结果表明，成功构建了靶向干扰 ppGalNAc-T2 基因表达的慢病毒载体，感染 Jurkat 细胞后能稳定干扰 ppGalNAc-T2 基因表达．MTT 和 Transwell 实验研究发现，下调 ppGalNAc-T2 基因表达对 Jurkat 细胞增殖和迁移有抑制作用．

关键词　ppGalNAc-T2，慢病毒，RNA 干扰，Jurkat 细胞，细胞增殖，细胞迁移

学科分类号　Q53；R34　　**DOI**: 10.3724/SP.J.1206.2011.00018

---

* 国家自然科学基金资助项目(30670462).

** 通讯联系人.

Tel: 0512-65880407, E-mail: wushiliang@suda.edu.cn

收稿日期：2011-01-10，接受日期：2011-03-22