The oncogenicity change and effect on tumor of HL-60 cells with silent nucleostemin gene in nude mice

FU Shuzhen [1], SUN Xiaoli[1], Abdallah Dlykan[1], JIA Yu[1], WANG Yuanyuan[2], LIU Shuai[1], YU Lina[3], ZHANG Hui[4], YUE Baohong[1]

1. Department of Laboratory Medicine, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan 450052, China
2. Department of Laboratory Medicine, The Affiliated Tumor Hospital (Henan Tumor Hospital), Zhengzhou University, Zhengzhou, Henan 450003, China
3. Department of Laboratory Medicine, The First People's Hospital of Zhengzhou City, Zhengzhou, Henan 450000, China
4. Department of Laboratory Medicine, Henan Medical College for Staff and Workers Zhengzhou, Henan 451191, China

ybh2002@163.com

Abstract: Objecttive. To investigate the oncogenicity change of HL-60 cells with silent nucleostemin gene in nude mice and the role of Nucleostemin（NS）specific short hairpin RNA （NS-shRNA）for the anti-leukemia effect in nude mice xenograft tumor model. Methods. HL-60 cells were taken as the model, and were directly transfected with one of Nucleostemin short hairpin RNA（NS-shRNA） which its effect of silencing NS gene is remarkable . In addition, negative control group and blank group were set up. The progress of tumors was observed regularly. Tissues of tumor in every group were handled with pathological section and dyed with HE. Determine the NS protein by immunocytochemisty. In addition, the heterogenic nude mice xenograft tumor models of high-oncogenic HL-60 leukemia cells were established. NS-shRNA was synthesized in vitro to prepare lipid inclusion, and was intraperitoneal inoculated into the mice. The volume and weight of the tumor bodys were measured, the slices of xenograft tumor were stained by HE dye, the NS protein inhibiting effect was detected by immunocytochemistry, and the apoptotic cells of HL-60 in the tumor body were examined by Tunel technique. Results. Different groups need different time to progress the tumor. The experimental group need longer than control group, and the tumor was smaller. The final tumor volume of mouse in experimental group was different significantly with other two groups（$P<0.05$）. But the difference between negative control group and blank group was not significant（$P>0.05$）. Under microscope, it showed that interstitial connective tissue and blood vessels were fewer than other two groups, and the cells arranged becomes loosely. HL-60 cells were not uniform. The cells with karyorrhexis and small nucleus increased. Pykno-levels of nuclear chromatin were not uniform and tumor giant cells decreased. All mice in our study were successfully transplanted by high-oncogenic HL-60 leukemia cells, and the volume of the tumors was even smaller. After treated with NS-shRNA lipid inclusion for 13 days, the tumor volume，weight and NS protein in the tumor cells were statistically lower than control groups. Large areas of patchy destroyed of tumor tissue and "apoptosis character" changes appeared in treated group. A great deal of apoptotic cells appeared in tumor tissue after therapy, detected by Tunel technique. Conclusion. The oncogenicity of HL-60 cells with silent nucleostemin gene was decreased. It is likely related to the change of cells′ biological characters. The anti-leukemia effect of NS-shRNA in nude mice xenograft tumor model is significant; one of the mechanisms probably induce the apoptosis of leukemia cells by the down-regulation of NS expression.

[FU Shuzhen, SUN Xiaoli, Abdallah Dlykan, JIA Yu, WANG Yangyuan, LIU Shuai, YU Lina, ZHANG Hui, YUE Baohong. **The oncogenicity change and effect on tumor of HL-60 cells with silent nucleostemin gene in nude mice** . Life Sci J 2012;9(3):226-232] (ISSN:1097-8135). http://www.lifesciencesite.com. 34

Keywords: Nucleostemin; short hairpin RNA; nude mice xenograft tumor; leukemia; HL-60 cell; oncogenicity

1. Introduction

Neucleostemin(NS), which was discovered in 2002,is a p53-binding protein predominantly exists in the nucleus of the stem cells, but not in terminally differentiated cells. The ensuing research found that nucleostemin was highly expressed in some kinds of human solid tumor or cancer cells [1, 2, 3]. The previous study of our team revealed that NS was also expressed continuously and highly in the leukemia cell lines HL-60 and K562, as well as the leukemia cells derived from patients who suffered from myeloid, monocytic and lymphoid leukemia. The further study found NS-specific short hairpin RNA (NS-shRNA) could interfere NS-mRNA and NS protein expression of the leukemia cells HL-60, and then affect its biological characteristics including proliferation, differentiation and apoptosis[4,5,6]. Based on the series of researches above, this study will change the researches focusing

on NS from in vitro to in vivo and from cellular level to histological level. We inoculated the NS-shRNA-treated HL-60 cells into the nude mice, and then observed its oncogenicity and tumorigenesis. In addition, we transfect NS-shRNA into nude mice xenograft tumor models as Lipid inclusions to study the effects to the growth and the indicator change of the tumor, and to probe into the antileukemic action of NS-shRNA in nude mice. This work would form the basis of understanding further the function of NS and changing the properties of tumor cells via inhibiting NS.

2. Materials and Methods

2.1 Materials and reagents

The human leukemia cell lines HL-60 cells were provided by Shanghai Institutes for Cell; Balb/c nude mice (SPF grade, male, 5~6w old and 18~22g) were purchased from the Slack experimental animals Co. Ltd. in Shanghai. Transwell was provided by Coster Co, Polycarbonate microporous membrane filter was provided by Watllman Co. Control sequence of siRNA was provided by shanghai Gema genepharma Co.ltd. immunocytochemistry kit was provided by zhongshan goldenbridge biotechnolocy Co. Tunel kit was provided by huamei biotechnolocy Co.ltd.

2.2 Experimental animals

Balb/c nude mice (SPF grade, male, 5~6w old and 18~22g) were purchased from the Slack experimental animals Co. Ltd. in Shanghai. The animals were kept in the laminar flow room of "isolated cage with independent air supply" in common sterilizing room. Enviromental conditions were controlled at 24~26℃ for temperature and 35%~45% for humidity. The cages, padding, water and fodder were all disinfected. The experimenter disinfected hands before conducting aseptic manipulation.

2.3 The sequences of NS-shRNA

According to the principles of shRNA designing, two NS specific shRNA which each contains a 9bp loop [aaguucucu] were finally determined. After annealing, the NS-shRNA could fold to the hairpin structure naturally. Preliminary experiments were carried out in order to select a more effective NS-shRNA for the follow-on experiments.

NS-shRNA- 1:

5′-GCUGAGCUAAGGAAACAGAucucuu

gaaUCUGUUUCCUUAGCUCAGCUU-3′

NS-shRNA-2:

5′-GCCUAGGAAAGACCCAGGAaaguuc

ucuUCCUGGGUCUUUCCUAGGCUU-3′

2.4 The sequences of negative control siRNA oligo

The sense strand:

5′ -UUCUCCGAACGUGUCACGUTT-3′

The antisense strand:

5′ -ACGUGACACGUUCGGAGAATT-3′

The negative control siRNA sequence was unrelated with NS, and had no homologous coding sequence with mammals. The 21bp sense strand contained a 19bp sequence which had nothing to do with NS-mRNA and was ended in a 2-thymidine 3′-overhang. The 19bp sequence of the sense strand and the antisense strand were complementary. This siRNA oligo sequence was synthesized by Shanghai Gema genepharma Co.ltd.

2.5 Proceeding preliminary experiment to select NS-shRNA

HL-60 cells were cultured routinely and harvested in logarithmic growth phase. Then the cells were adjusted to the density of 4×10^5/ml with whole medium and aliquoted into 6-well plates for 2.5ml per well. These cells were divided randomly into the transfected groups (R1 and R2) and the control groups (C1 and C2). The steps of transfection:

7.5μl Code Break siRNA transfection reagent was added to 625μl non-serum medium. R1 group was added NS-shRNA-1,and R2 group was added NS-shRNA-2, the final concentration was adjusted to 10 nmol/L. C1 group was added transfection reagent only as blank control, and C2 was added non-related siRNA sequence as negative control, the final concentration was also 10 nmol/L. 48 hours after transfection, the total RNA of each group were extracted by TRIzol reagent for NS gene amplification by RT-PCR, in order to detect the blocking effect of NS-mRNA.

2.6 Preparation for inoculation

24 hours before inoculation, the closed laminar flow chambers of the "independent air supply isolation cages" were placed under the ^{60}Co radiation therapeutic machine, giving a total dose of 4.5Gy exposure to the mice inside the cage. HL-60 cells were cultured in 200ml culture flask and then were transfected. These cells were divided into 3 groups:

The transfected group: Select the more effective NS-shRNA to transfect HL-60 cells and then inoculate.

The negative group: Transfect the HL-60 cells with non-related siRNA and then inoculate.

The blank group: Add the transfection reagent only.

The experimental mice were grouped randomly, and each group had 8 mice.

2.7 The mice were inoculated by the transfected HL-60 cells

24 hours after transfection, each group cells were harvested and centrifuged at 500rpm for 10min. Then the cells were collected to a sterile 40ml centrifuge tube and washed with sterile saline. The cells were

resuspended for cell counting and the cell concentration was adjusted to 1×10^8/ml, then the cells were inoculated subcutaneously into the front leg of nude mice at a dose of 0.25ml (containing 2.5×10^7 cells). Aseptic operation was followed in the whole procedure.The nude mice were observed continuously for 5 days and the tumor formation was recorded. 5 weeks later, the nude mice were sacrificed by stretching neck method and dissected, then the tumor tissues were removed and fixed by paraformaldehyde(PFA) for pathological section and HE staining. The C1 group and C2 group were inoculated and handled with the same methods as above.

2.8 Determination of NS protein-positive cells

The cells were identified through immunocytochemistry staining under optical microscope. The cells which showed brown-yellow or brown blocky materials in the nuclei or nucleolus were positive.

2.9 Establish the heterogeneic athymic mice xenograft tumor models of high-oncogenic HL-60 leukemia cells

Collect HL-60 cells in doubling generation time and adjust the concentration, then inject hypodermically the cells into dorsal part of foreleg on mice which was exposed to ^{60}Co with 4.5GY. After the tumor grow to enough big, cut off the cells and culture the cells in vitro to fifth generation. Add 0.5ml liquid supernatant of HL-60 cells cultured without serum for 24h into TCPS, and add 25ul suspension cells into inside Transwell, adjusting the cells to density to 2×105/ml and culture for 12h. With giemsa staining, select 5 fields of view presently by microscope to count the cells and the mean value representing the cell's oncogenicity. Inject the cells into 30 mices and divide them into 3 groups presently when the tumors grow to 400mm^3.

2.10 Preparing the lipid inclusions of NS-shRNA and control siRNA

Inject 125μl cultured medium without serum and antibiotic into 1.5ml centrifuge tube, and mixing after adding 3.0ul transfection Reagent. Then culture at room temperature for 15~20min.

2.11 Inoculating the NS-shRNA and control siRNA into mice

Every mouse of treatment group was inoculated lipid inclusions of NS-shRNA with 3μg NS-shRNA every 3 days for 4 times. Every mouse of negative control group was inoculated control siRNA with the same method. Every mouse of blank control was inoculated normal saline. After 13 days, kill the mice and get the tumor of every group to weigh and measure the volume of the tumor.

2.12 Observation of the tumor formation

The tumor formation in nude mice was observed regularly. When the tumor grew out, the tumor volume was measured by vernier caliper every 3 days, including the maximum diameter (Max) and minimum diameter (Min) of the tumor. Then the tumor volume was calculated and the tumor growth curve was mapped. The formula for calculating the tumor volume is as follows:

Tumor volume(V) =Maximum diameter(Max)×Minimum diameter square(Min ^{2})/2[7].

2.13 Observation of the histological sections

Sections and regions which were dyed well and uniformly were selected to observe the cell size, the connective tissue, the distribution of blood vessels, the nuclei size and the chromatin.

2.14 Text the apoptosis of tumor by Tunel

The tumor with embedded in paraffin and serial section was cultured with 0.3%H_2O_2 carbinol for 30min, washed by water three times and then ice-bathed with connect fully liquid for 2min, according to the Tunel kit.

2.15 Statistical analysis

All data were processed by SPSS 16.0 and reported as $\bar{x} \pm s$. Statistical significance was set P<0.05, and P<0.01 means high significance.

3. Results

3.1 Detection of blocking effect of the synthetic NS-shRNA

HL-60 cells were harvested for NS gene amplification by RT-PCR after incubated with the two synthetic NS-shRNA repectively. According to scanning gray scale analysis, the related score is 0.826 in C1 group, 0.809 in C2 group, 0.503 in R1(NS-shRNA-1) group and 0.207 in R2(NS-shRNA-2) group. Thus, the synthetic NS-shRNA-1 and NS-shRNA-2 could block the expression of NS-mRNA, with the inhibiting rates of 39.10 % and 74.94 %. It showed that NS-shRNA-2 was more effective, so it was selected for further experiments (Figure 1).

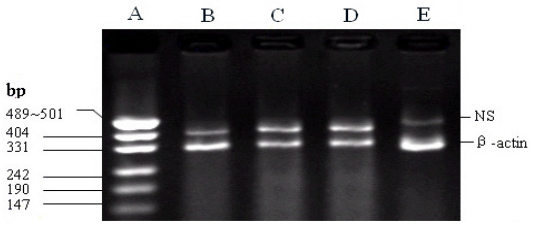

Fig 1 RT-PCR result of human leukemia HL-60 cells treated 48 h by NS-shRNA

A：DNA Mark B： NS-shRNA-1

C：blank control D： negative control

E： NS-shRNA-2

3.2 The tumor formation of HL-60 cells transfected with NS-shRNA-2 in nude mice

The mice in C1 group and C2 group began to grow tumors at about 14~15 day after inoculated with HL-60 cells, but the tumor formation of the mice in R2 group began at about 18~19 day after inoculation. The time of tumor formation in experimental group (R2 group) was longer than the control groups (C1 and C2 group). Finally, there were 4 mice in each of the three groups (4/8) formed the tumors.

3.3 The change of NS protein in HL-60 cells after transfection

72 hours after HL-60 cells incubated with NS-shRNA-2, the immunocytochemistry staining showed that NS expression rate of the cells in C1 group and C2 group were（94.77±2.69）% and（93.55±2.84）%, and the positive score were 180.22±16.41 and 177.33±13.34,respectively. NS protein expression of the HL-60 cells in R2 group were significantly decreased, the expression rate was（46.33±2.67）% and the score was 51.33±6.23. The difference of the score between the two control groups(C1 and C2) was not significant(P>0.05), but comparing R2 group with the two control groups(C1 and C2), it was significant. The results showed that the synthetic NS-shRNA-2 in vitro could obviously inhibit the expression of NS protein in HL-60 cells, and the inhibition rate reached to 71.5 %(figure 2 and 3). It indicated that after NS-shRNA-2, which entered into HL-60 cells by transfection, inhibited part of the expression of NS-mRNA, the amount of NS protein could decrease.

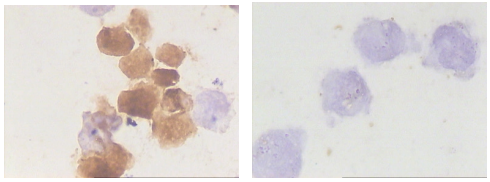

Fig 2 Detecting NS protein by immunocytochemistry in HL-60 cells transfected

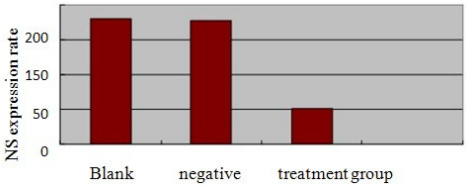

Fig 3 The expression of NS protein in HL-60 cells by immunocytochemistry after transfected

3.4 The tumor growth after the HL-60 cells tranfected with NS-shRNA were implanted into the nude mice

After implantation, the tumor growth was observed regularly, and the tumor size was measured every 3 days. 30 days later, the final size of the tumor was calculated, and the tumor was weighed. In appearance, the tumor size of the R2 group was obviously smaller than the two control groups (C1 and C2). The stripped tumor was consistent with the tumor in vivo (figure 4,5). Comparing the R2 group with the C1 and C2 group, the tumor size and weight were significantly light(P<0.05); There were no significant differences between the C1 and the C2 group(P>0.05)（table 1, figure 6）.

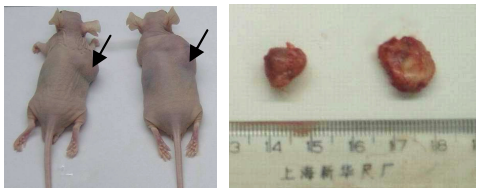

Fig 4 the growth of tumor in NS-shRNA-2 group nude mice and tumor bodies
（The arrows indicate the tumors）

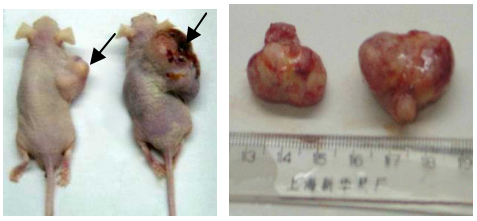

Fig 5 the growth of tumor in control group nude mice and tumor bodies
（The arrows indicate the tumors）

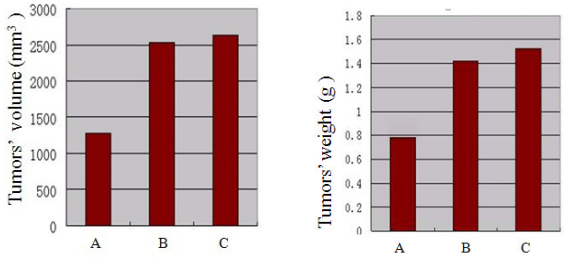

Fig 6 the tumors volume and weight
A: the transfected group B: the negative control group C: the blank control group

3.5 The histological observation of the leukemia xenotransplantation tumors after transfection with NS-shRNA

30 days later, the nude mice were all sacrificed for dissected examination, and no metastatic lesions were found in lymph nodes or organs. The tumor tissues derived from each group were embedded in paraffin for making pathological sections and then stained with HE. Under the microscope, it was observed that the cells in tumor tissues obtained from the three groups were all grew focally. The cells of the C1 and C2 group were similar, they were arranged tightly with a lot of connective tissues and vessels; the HL-60 cells in the

tumor tissues were varied in size, the chromatins were condensed and heave stained, the tumor giant cells were seen often, and the pleomorphism was marked, the apoptotic cells were occasionally found. The connective tissues and vessels of the R2 group were less than the C1 and C2 group, the cells in the tumor tissues were arranged loose, leaving much space; the sizes of HL-60 cells varied much greatly, nuclear fragmentations and small nuclei cells increased, the density of the chromatin was inhomogeneous, and the tumor giant cells were less seen (figure 7).

Table 1. The growth of tumors after transfected by NS-shRNA-2 （$\bar{x} \pm s$）

group	Treated	Negative control	Blank control
Inoculation num (n)	8	8	8
Tumor num (n)	4	4	4
rate	50%	50%	50%
Volume (mm^3)	1282.6±434.1#	2533.3±683.1	2632.5±621.8
weight(g)	0.79±0.29*	1.42±0.4 1	1.53±0.37

#Compared to other two groups: t=3.090、3.560, both P<0.05
Compared between two control group: t=0.304, P>0.05
* Compared to other two groups: t=2.509、3.148, both P<0.05
Compared between two control group: t=0.398, P>0.05

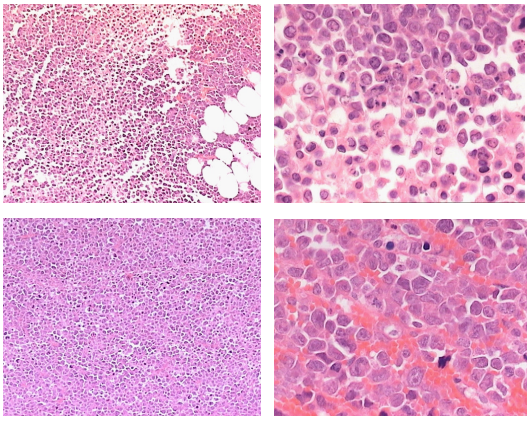

Fig 7 The tumor tissue of transfected group nude mice(up) and contral group nude mice(down) HE staining Left：200× Right：400×

3.6 Establish the nude mice xenograft tumor models of high-oncogenic HL-60 leukemia cells

The mice exposed to ^{60}Co appeared tumor after 7 days by inoculated with HL-60 cells. The ratio of oncogenicity is 70%. Get the tumor and washed to collect the cells after 10 days. After 5 passage, testing the cell's nvasiveness by Transwell test indicate that there have obvious differention about the cell's nvasiveness between the treated with NS-shRNA and not. Cells can cross the membrane is 216±12.3, and the cells can't cross is 159±20.7. P<0.05 by t test. 30 mice was inoculated by HL-60 cells for the second time and appeared tumor with same volume. After 15 days, the trement group is （407.8±45.3）mm^3，the negative control group is （415.7±61.0）$mm^3$and the blank control group is （413.2±75.3）mm^3. The difference among the three groups was not obvious processed by Statistics.

3.7 The antileukemie effection of NS-shRNA in mice

Injected with HL-60 cells 15 days after, inoculated NS-shRNA-2 into treatment groups. After 13 days, the tumor of this group grows with rate of 207.1%. While the negative control group is 497.1% and the blank control group is 569.6%. The tumor's volume of the three groups as the table 2.

Table 2. Stsatistic results of tumors volume and weight

Group	Treatment	Negative contrc	Blank control
Volume before treatment（mm^3）	407.8±45.3#	415.7±61.0	413.2±75.3
Volume after treatmer（mm^3）	1252.4±348.7	2472.0±279.5	2766.7±369.2
Volume increase（%）	207.6	494.7	570.0
Weight（g）	1.18±0.27*	2.04±0.73	2.35±0.41

#vs negative control, blank control respectively, both P>0.05; & vs negative control, blank control respectively, both P<0.01; * vs blank control, negative control respectively, both P<0.01; And P>0.05 compared with negative control and blank control.

3.8 The result of histotomy and HE staining

Observed by microscope, the cells in tumor of two control groups arrayed in order, and there have much more connective tissues and vessels in interstitial than the trentment group. The cells in treatment group's cells become different in volume and form. Many cells have some characteristic of apoptosis, such as become smaller, shrinking, nuclear chromatin Agglutination and break into rounded granule like apoptotic body (Figure 8). The tumors have many broken areas.

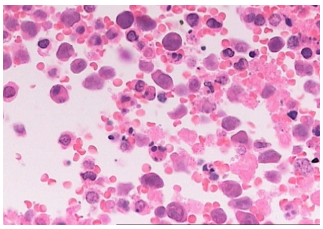

Figure 8. Tumor tissue treated by HE staining（400×）

3.9 The result of immunocytochemistry of the NS protein in cells

The positive rate of NS protein in HL-60 cells of the tumor of blank control group is （97.33±1.76）%, the scores is 220.93±16.54, while the negative control group is（90.72±1.47）% and 195.11±9.71. the positive of NS protein of treatment group is lower than other two groups with (71.59±1.80）% and 110.26±13.46. it indicated that there is obvious difference between the treatment and other two groups($P<0.01$). The two control groups have no obvious difference($P>0.05$). The restrain rate of NS protein in treatment group is 43.5% and 50.1%. (Figure 9)

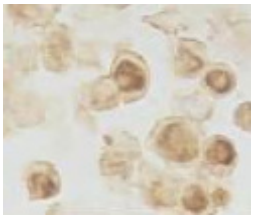

Figure 9. Immunohistochemistry results of NS protein in treated tumor tissues（1000×）

3.10 The result of tunel test

There are many apoptotic cells in the tumor of treatment, while the tumor in control groups have little apoptotic cells. (Figure 10)

A B C

A：Treated group； B：Negative control group； C：Blank control group

Figure 10. Tunel results（400×）

4. Discussion

Nucleostemin is a kind of protein that is necessary for maintaining the proliferation of cells and makes the cells stop differentiation [8]. NS protein may be a specific regulatory factor responsible for stem cells and cancer cells crossing G2/M checkpoint [9]. It has been demonstrated that the down-regulation of NS expression could result in cell cycle arrest and cell differentiation. At present the consensus made by domestic and international experts is that nucleostemin is highly expressed in stem cells and cancer cells. It is considered that NS is related to the regulation of stem cells and cancer cells proliferation, and it could reflect the situation of cell proliferation [10~12]. Some scholars have seen NS as a marker of stem cells and a target of anticancer drugs [11, 12]. Previous studies of our research group have demonstrated that NS-shRNA could interfere the expression of NS protein, and then influence the biological characteristics of HL-60 cells including proliferation, differentiation and apoptosis [6, 13].

Silencing NS gene could obviously weak the oncogenicity of HL-60 cells. The tumor formation rate from the point of view, the time of transfected group is longer than the control group, and the tumor size and weight are all less than the control group. There are also significant differences in stromal connective tissues, blood vessels and HL-60 cells between the transfected group and the control group, this indicates that silencing the NS gene by NS-shRNA-2 could weaken the oncogenicity of HL-60 cells.

The direct transfection RNAi without building the expression vector is a transient response. Along with the extending of time, the interfering effect of NS-shRNA will gradually disappear. At the initial stage of HL -60 cells are inoculated into the nude mice, the interfering effect of NS-shRNA-2 weakens the cell proliferation and reduces the number of cells which could form the tumor, further studies need to be done to determine whether cell differentiation and apoptosis are accompanied in this stage, so we consider that's why the transfected cells grew smaller tumors and need longer time.

The success rate of tumorigenesis of various types of leukemia cells in nude mice is very low. The reason is that the NK cell activity in thymus defecting nude mice is high; however, most leukemia cells are sensitive to NK cells [14]. We gave the nude mice a total dose of 4.5Gy exposure to $^{60}C_O$ radiation before inoculating the leukemia cells, in order to reduce the immune response of nude mice and increase the success rate of transplanted tumors. NS is a p53-binding protein, its biological functions needs the mediation of p53. The HL-60 cells used in this study are p53-null, so we speculate that NS should have another signal pathway except p53, which is the next target of our research.

In this study, the synthesis of the NS- shRNA have been transferred in the form of lipid inclusions in tumorigenic nude mice , and effectively blocked the NS protein expression of the HL-60 leukemia cells in the tumor tissue .Transplanted tumor volume and weight were significantly less than the two control groups. Histological observation of structural damage

to the tumor tissue, cell lyses, more cells have the characteristics of “apoptosis". Tunel confirmed that apoptotic cells of the treatment groups were significantly increased. Therefore, this study suggests that the NS -shRNA played an anti-leukemia effect in nude mice, this effect may be achieved through regulate NS protein expression of the leukemia cells .Tsai and Liu et al used solid tumor cells and found that the NS product's inhibition accompanied by diminishing of cell proliferation capacity, and part of the cancer cells exit the cell cycle [15]. We previously using synthetic NS - shRNA in vitro studies acted on leukemia cells, it also have similar results, which suggests that the diminished capacity of cell proliferation in this case may be related to enhancement of cell apoptosis. Therefore induced apoptosis making leukemia cell proliferation diminish may be an important way of vivo anti- leukemia mitigation in animal model of nude mice. It laid a theoretical foundation for the NS gene as candidate genes for cancer treatment, Nikpour P recently get results similar to the experimental and perspectives [16].

Stephanie Filleur studied siRNA as a therapeutic drug into the animals, and the result showed that intraperitoneal injection is the best way [17]. This study also confirmed the intraperitoneal injection is effective, and intraperitoneal injection as a treatment model , especially used in people malignant tumors and diffusion growth of malignant tumors, is more easily accepted .However, it is most worth considering that whether this treatment model is also effective in the case of the perfect human body immune mechanism. If it can be proved to be the same or similar effective, it will play a tremendous role in RNAi-based gene therapy.

On the mechanism of NS expression inducing leukemia cell apoptosis, the signal transduction pathway in this process, shRNA in nude mice is how to move to the tumor tissue, and a series of questions also require further study.

Corresponding Author：
YUE Baohong
Department of Laboratory Medicine
The First Affiliated Hospital, Zhengzhou University
Zhengzhou, Henan 450052, China
E-mal: ybh2002@163.com

References

1. Li J, Li YL. Progress in the research of nucleostemin in tumors[J]. Tumor, July, 2010, V o. l 30, No. 7: 631-633.
2. Yang JF, Zhang H, Wu YD, et al. Expression of nucleostemin gene in prostate cancer and its correlation with prostate specific antigen [J]. Acta Academiae Medicine Militaris Tertiae, 2008 ; 30 (3) : 244-246
3. Li J, Li YL, Cheng X. Expression and significance of nucleostemin gene in non-small cell lung cancer [J]. Chinese Journal of Histochemistry and Cytochemistry, 2010, 19(3):229-233.
4. Yue BH, Wang YY, etal. Effects of knocking-down Nucleostemin gene on apoptosis of HL-60 cells in vitro. Life Science Journal, 2011;8(3):290~294.
5. Yue BH, She HC, Wang QX, et al. Detection of effects of nucleostemin gene silencing on morphology and cytochemistry of HL-60 cells in vitro [J]. Laboratory Medicine, April 2009,24（4）：281-85.
6. Yue BH, Yu LN, Wang YY. Effects of Silent Nucleostemin Gene Expression on Apoptosis of HL-60 Cells In Vitro [J]. Journal of Experimental Hematology，2009，17(2): 319- 323
7. Sun. Y，Kim. H，Parker. M，et al. Lack of suppression of tumor cell phenotype by overexpression of TIMP-3 in rat JB6 tumor cells identification of a transfectant with increased tumorigenicity and invasiveness [J]. Anticancer Res，1996，16（10）:1-7.
8. Ran L, Xu Yong. Advance in nucleostemin research. Journal of Tianjin Medical University, 2007, 13 (1):132-134.
9. Tsai R Y L, Mckay R D.A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells [J]. Genes Dev, 2002, 16（23）: 2991-3003.
10. Lin T,Meng L, Li Y,Tsai RY.Tumor-initiating function of nucleostemin-enriched mammary tumor cells[J]. Cancer Res November 15,2010, 70:9444-9452.
11. Dorothy Lo and Hua Lu.Nucleostemin: Another nucleolar “Twister” of the p53-MDM2 loop .Cell Cycle[J]. 2010 August ; 9(16): 3227–3232.
12. Nikpour P, Mowla SJ, Jafarnejad SM, etal. Differential effects of Nucleostemin suppression on cell cycle arrest and apoptosis in the bladder cancer cell lines 5637 and SW1710[J]. Cell Prolif. 2009, 42(6):762-769.
13. Yue BH, Zhu XY, Zhang GY, et al. Effect of specific short hairpin RNA of nucleostemin on differentiation antigens and biological properties of HL-60 cells. Acta Academiae Medicine Militaris Tertiae，2006,28（11）：1191-1194.
14. Ono A, Amos DB, Koren HS. Selective cellular natural killing against human leukaemic T cells and thymus. Nature, 1977, 266(5602):546-548.
15. Liu SJ, Cai ZW, Liu Y, etal. Role of nucleostemin in growth regulation of gastric cancer, liver cancer and other malignancies [J]. World J Gastroenterol, 2004, 10(9): 1246 – 1249.
16. Nikpour P, Mowla SJ, Jafarnejad SM, et al. Differential effects of Nucleostemin suppression on cell cycle arrest and apoptosis in the bladder cancer cell lines 5637 and SW1710[J]. Cell Prolif. 2009, 42(6):762-769.
17. Filleur S, Courtin A, Ait-Si-Ali S, et al. SiRNA-mediated inhibition of vascular endothelial growth factor severely limits tumor resistance to antiangiogenic thrombospondin-1 and slows tumor vascularization and growth[J]. Cancer Research，2003, 63(14):3919~3922.

5/08/2012