Clinical Study

Interferon Alpha on Expression of hTERT mRNA in Peripheral Blood Mononuclear Cells of Patients with Chronic Hepatitis B

Chuan-wu Zhu,[1] Ming Chen,[2] Xiang-rong Luo,[1] Hai-yan Wang,[1] Li-hua Wang,[2] Jian-hong Wu,[1] Ming Li,[1] Xue-hua Zhang,[1] Wei Zhu,[1] Jian-zhong Ye,[1] and Feng Qian[1]

[1] *Department of Hepatology, The Fifth People's Hospital of Suzhou, Suzhou 215007, China*
[2] *Department of Infectious Diseases, Affiliated Hospital of Medical College of Xuzhou, Xuzhou 221000, China*

Correspondence should be addressed to Chuan-wu Zhu, zhuchw@126.com

Cell division is closely related to telomerase activity (hTERT mRNA). Lower expression of lymphocitic hTERT mRNA may easily cause cell aging, which is not beneficial to maintaining a durable lymphocyte division. To date, there is no study to investigate IFNα therapy on hTERT mRNA expression in PBMCs of patients with chronic hepatitis B (CHB). We quantitatively detected hTERT mRNA from study subjects and made each hTERT mRNA normalized (NhTERT mRNA). Mean NhTERT mRNA level was lower in either CHB group, but it significantly increased in IFNα-treated group compared with CHB control group, and a longer duration of IFNα therapy could increase the level. Moreover, the mean NhTERT mRNA in subgroup with HBeAg loss was significantly higher than that in subgroup without. NhTERT mRNA was markedly correlated with CD3$^+$ T lymphocyte count and CD4$^+$/CD8$^+$ ratio. The results showed that IFNα therapy could upregulate the expression of hTERT mRNA in PBMCs.

1. Introduction

Telomeres are the terminal repeats of the chromosomes in eukaryotic cells and continuously shorten with cell division. Acting as a molecular clock, telomeres control the capacity of cell division and have a close relationship with cell senescence [1]. Telomerase is an RNA-dependent DNA polymerase, maintains the telomere length, and makes the cell achieve unlimited proliferation capacity [2]. Telomerase consists of two subunits, namely, functional RNA component (human telomerase RNA, hTR) and human telomerase reverse transcriptase catalytic subunit (hTERT) [3, 4]. hTR, which is highly expressed in all tissues regardless of telomerase activity, provides telomerase with the template for the synthesis of telomeres, while the expression of hTERT is in accordance with telomerase activity [5, 6]. Therefore, the level of hTERT mRNA could reflect the activity of telomerase. Germ cells and tumor cells often express higher level of telomerase activity, whereas most of normal somatic cells do not [7, 8], indicating that telomerase expression is closely related to the cell differentiation and development. The lymphocytes among somatic cells, however, can produce comparatively higher level of telomerase activity [9, 10], which can keep telomere length of lymphocytes from shortening, and thus play an important role in maintaining a durable cell differentiation. If the lymphocytes express low level of telomerase activity, telomere length will gradually shorten with cell division and finally result in cell aging and immune dysfunction, which has been confirmed in the researches of human immunodeficiency virus (HIV) and chronic hepatitis B virus (HBV) infection [11, 12]. Up to now, little is known about the alteration of telomerase activity in lymphocytes during the antiviral therapy on patients with chronic hepatitis B (CHB). We herein quantitatively detected the expression of hTERT mRNA in peripheral blood mononuclear cells (PBMCs) from CHB patients who received interferon alpha (IFNα) treatment and attempted for the first time to study the possible relationship between the immunomodulator and the telomerase activity in PBMCs.

2. Materials and Methods

2.1. Study Subjects. All investigated CHB patients, who met the criteria of the guidelines of prevention and treatment for chronic hepatitis B issued by Chinese Society of Hepatology [13], were those hospitalized in the Fifth People's Hospital of Suzhou during the year of 2009. The study was composed of three groups, namely, IFNα-treated group, CHB control group, and healthy control group. None of the patients in IFNα-treated group and CHB control group received any immunomodulator treatment including immune enhancers or immunosuppressive agents, as well as antiviral agents including nucleoside/nucleotide analogues for at least 6 months before IFNα therapy initiated. All patients were ruled out the coinfection with hepatitis A, C, D, or E viruses, as well as other liver damages caused by alcohol, drug, autoimmunity, and so forth. Healthy control group consisted of our 10 volunteer colleagues, who had normal liver function and were tested negative for the serum immunological markers of hepatitis B, C, and D virus infection. All subjects gave their voluntary informed consents about the study, and the research protocol was approved by the ethics committee of the Fifth People's Hospital of Suzhou. In IFNα-treated group, all the patients were given conventional IFNα-2b 5 MU intramuscular injection once a day for 2 weeks, and then were injected every other day for the total duration of 1 year. During the treatment, liver function and blood cell counts were tested monthly, and HBV serological markers and HBV DNA load were tested every 3 months.

2.2. Baseline Clinical Tests. In IFNα-treated group and CHB control group, all the baseline HBV serological markers (Abbott Ireland, Diagnostics Division, Sligo, Ireland), HBV DNA loads (Shanghai Shenyou Technology Co. Ltd, Shanghai, China), HBV genotypes (Shanghai ZJ Biological Technology Co. Ltd, Shanghai, China), and the routine biochemical tests, including liver function, kidney function, thyroid function, blood sugar, and peripheral blood cell counts, and so forth were detected. Lymphocyte subsets (BD Biosciences, San Jose, CA, USA) in 28 cases of IFNα-treated group and 26 cases of CHB control group were detected at baseline, and the lymphocyte subsets were retested in 26 patients after given IFNα treatment for at least 6 months.

2.3. PBMCs Sample Collection. 5 mL of heparin-anticoagulated peripheral blood was collected from each subject. Among them, the samples from IFNα-treated group were collected at the time of 6, 9, and 12 months of treatment, and those from CHB control group were collected on the second day of the patient's hospitalization. PBMCs were isolated by standard Ficoll density-gradient centrifugation and then washed with 0.01 M phosphate buffer solution (PBS). After centrifugation, about 5×10^6 cells were gathered for each sample and kept frozen at $-80°$C until the samples were used.

2.4. Total Cellular RNA Extraction. Total cellular RNA was, respectively, extracted using BIOZOL (Hangzhou Bioer Technology Co. Ltd, Hangzhou, China) according to the manufacturer's instruction. Briefly, each PBMCs sample was, respectively, precipitated and lysed, and then the cellular RNA was isolated, precipitated, washed, and finally dissolved in 30 μL of RNase-free water. The integrity of RNA was determined, respectively. The samples with obvious 28S and 18S rRNA components were used for the following study.

2.5. Detection of hTERT mRNA and β-actin mRNA. hTERT mRNA was quantitatively detected by real-time reverse transcription polymerase chain reaction (RT-PCR) (AB Applied Biosystems 7300 real-time PCR System, USA), using the quantification kit (Hangzhou Bioer Technology Co. Ltd, Hangzhou, China) conducted in accordance with the product instruction. Total reactive volume was 40 μL and contained 4 μL of RNA sample (1 μg or so). After 40 cycles of reaction, the quantitative results could be obtained automatically from PCR detection system, and the unit of hTERT mRNA is copies/ml in terms of the kit indication. In order to exclude all the possible factors influencing the comparison of hTERT mRNA levels among different samples, β-actin was used as internal control and its mRNA was also quantitatively detected by real-time RT-PCR for each sample, using the quantification kit (Shanghai Genepharma Co. Ltd, Shanghai, China) performed according to the manufacturer's instruction. The results also could be obtained automatically and the unit is copies/μl. Therefore, a normalized hTERT mRNA (NhTERT mRNA) could be obtained for each sample; that is, NhTERT mRNA could be calculated as the copies of target gene hTERT mRNA divided by the copies of its internal control gene β-actin mRNA.

2.6. Statistical Analysis. A descriptive analysis of the variables collected from each subject was performed. Continuous variables were shown as median values and the dispersion measurement as range values. The chi-squared test and t-test were used for analysis of the selected variables. Mean NhTERT mRNA level was presented as mean $\pm$ SD. The differences between two groups were compared using independent-samples t-test and multiple groups using one-way analysis of variance. Spearman rank correlation analysis was used for bivariate correlation analysis. A probability value was determined using two-sided statistical test. $P < .05$ was considered to be statistically significant. All statistical analyses were performed using SPSS version 13.0 for windows (SPSS, Chicago, IL, USA).

3. Results

3.1. Baseline Characteristics of the Study Groups. The clinical characteristics of the three groups were listed in Table 1. Gender and age were listed for all groups. HBeAg status, aniline aminotransferase (ALT) levels, HBV DNA levels, and HBV genotypes were listed for all CHB patients. The counts of CD3 and the ratio of CD4$^+$/CD8$^+$ were listed for 28 patients in IFNα-treated group and 26 patients in CHB control group. There was/were no significant difference(s) for these baseline characteristics among the study groups.

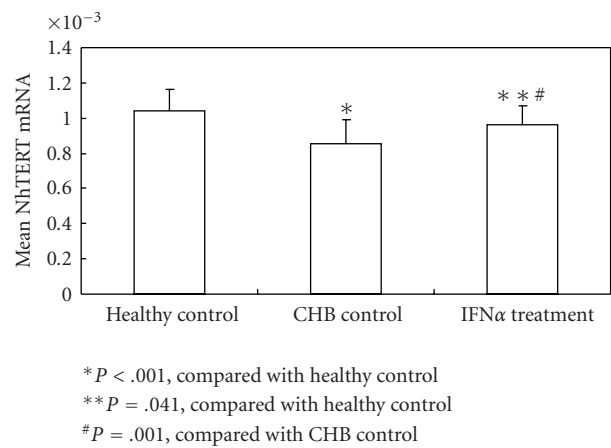TABLE 1: Clinical baseline characteristics of the study groups.

	IFNα-treated Group (n = 37)	CHB control group (n = 36)	Healthy control group (n = 10)	P-value
Male	25 (67.6)	28 (77.8)	6 (60)	.56
Median age in year (range)	27.3 (20–48)	28.6 (23–55)	30.6 (24–45)	.68
No. of HBeAg + patients	31 (83.8)	25 (69.4)	N/A	.65
Median ALT (range), IU/L	148.3 (83–288)	132.8 (83–352)	N/A	.61
Median HBV DNA load (range), log copies/ml	7.6 (5.2–9.4)	7.2 (4.8–8.7)	N/A	.72
HBV genotype (no. of patients)	Type B (11)	Type B (8)		.47
	Type C (23)	Type C (25)	N/A	.51
	Other (3)	Other (3)		.97
Median CD3 count (range), 10^6 cell/L	1138 (971–1493)	1278 (856–1387)	N/A	.72
Median ratio of CD4/CD8 (range)	1.72 (1.13–2.57)	1.58 (1.26–2.63)	N/A	.51

Note. Date are no. (%) of patients, unless otherwise indicated; HBeAg+: positive for hepatitis B e antigen; ALT: alanine aminotransferase; HBV: hepatitis B virus; N/A, not applicable.

3.2. IFNα Therapy and Responses.

In IFNα-treated group, the results of change in biochemical, immunological, and virological indices were evaluated after the beginning of antiviral therapy for at least 6 months. In this study, there were 11 patients evaluated at the time of treatment for 6 months, 14 patients for 9 months, and 12 patients for 12 months. In the beginning of IFNα therapy, the varying degrees of flu-like symptoms occurred, the count of neutrophils and platelets decreased in some patients, and no severe adverse events which led to IFNα dose reduction or antiviral interruption happened. The biochemical, virological, and immunological responses to IFNα were as follows: ALT normalization (<40 IU/mL) in 23 cases, ALT elevation (≥40 IU/mL) in 14 cases; HBV DNA load <3 log/mL in 18 cases, HBV DNA load ≥3 log/mL in 19 cases; and among 31 cases of HBeAg positive patients, 14 cases with HBeAg loss, and 17 still with positive serum HBeAg.

3.3. Comparison of Mean NhTERT mRNA Levels.

Each level of NhTERT mRNA in PBMCs of the three groups could be seen in Table 2. Among the three groups, the mean NhTERT mRNA was 1.042×10^{-3} in healthy control group, 0.959×10^{-3} in IFNα-treated group, and 0.857×10^{-3} in CHB control group, respectively. The compared results were shown in Figure 1. There were no remarkable differences between different gender groups, genotype groups, as well as HBeAg positive and negative groups ($P = .753$, $P = .664$, and $P = .587$, resp.). Regarding the duration of IFNα treatment on the influence of mean NhTERT mRNA levels, Figure 2 showed that the patients with longer duration of IFNα treatment had higher levels of NhTERT mRNA in PBMCs.

3.4. Relationship of NhTERT mRNA with Clinical Characteristics.

Spearman rank correlation analysis showed that the NhTERT mRNA expression was not associated with age in

$*P < .001$, compared with healthy control
$**P = .041$, compared with healthy control
$\#P = .001$, compared with CHB control

Comparison of Mean NhTERT mRNA levels in the study groups.

	No.	Mean NhTERT mRNA level ($\times 10^{-3}$)	t- value	P- value
Healthy control group	10	1.042 ± 0.118		
CHB control group	36	0.857 ± 0.131	4.028	<.001*
IFNα treated group	37	0.959 ± 0.109	2.107	.041**
			3.605	.001#

*compared with healthy control group; **compared with healthy control group, and #compared with CHB control group.

FIGURE 1: Mean NhTERT mRNA levels in the study groups.

all subjects ($r = -.059$, $P = .594$, $n = 83$), and neither was with ALT in all CHB patients ($r = -.116$, $P = .329$, $n = 73$). There was significant relationship between the expression of NhTERT mRNA and the count of CD3+ T lymphocytes ($r = .304$, $P = .026$, $n = 54$) as well as the ratio of CD4+/CD8+ ($r = .285$, $P = .041$, $n = 54$) when the 54 patients with lymphocyte subset test were taken into

TABLE 2: NhTERT mRNA levels in PBMCs of all investigated subjects.

No.	IFNα treated group ($n = 37$) NhTERT mRNA	CHB control group ($n = 36$) NhTERT mRNA	Healthy control group ($n = 10$) NhTERT mRNA
1	0.911×10^{-3}	0.775×10^{-3}	1.094×10^{-3}
2	0.985×10^{-3}	0.930×10^{-3}	1.204×10^{-3}
3	1.016×10^{-3}	0.901×10^{-3}	1.017×10^{-3}
4	0.985×10^{-3}	0.673×10^{-3}	1.260×10^{-3}
5	1.096×10^{-3}	0.792×10^{-3}	0.959×10^{-3}
6	1.103×10^{-3}	0.885×10^{-3}	1.017×10^{-3}
7	0.990×10^{-3}	0.693×10^{-3}	0.902×10^{-3}
8	0.989×10^{-3}	0.989×10^{-3}	1.069×10^{-3}
9	1.002×10^{-3}	0.583×10^{-3}	0.992×10^{-3}
10	1.000×10^{-3}	0.841×10^{-3}	0.906×10^{-3}
11	0.983×10^{-3}	0.749×10^{-3}	
12	1.058×10^{-3}	0.927×10^{-3}	
13	0.914×10^{-3}	0.980×10^{-3}	
14	0.994×10^{-3}	0.736×10^{-3}	
15	0.989×10^{-3}	1.010×10^{-3}	
16	1.034×10^{-3}	0.875×10^{-3}	
17	1.042×10^{-3}	0.757×10^{-3}	
18	0.991×10^{-3}	0.954×10^{-3}	
19	1.061×10^{-3}	0.907×10^{-3}	
20	1.220×10^{-3}	1.001×10^{-3}	
21	0.924×10^{-3}	0.880×10^{-3}	
22	0.952×10^{-3}	0.887×10^{-3}	
23	0.994×10^{-3}	1.088×10^{-3}	
24	0.800×10^{-3}	0.790×10^{-3}	
25	0.980×10^{-3}	0.708×10^{-3}	
26	1.018×10^{-3}	0.941×10^{-3}	
27	0.809×10^{-3}	0.917×10^{-3}	
28	0.967×10^{-3}	0.655×10^{-3}	
29	1.088×10^{-3}	0.593×10^{-3}	
30	0.791×10^{-3}	0.916×10^{-3}	
31	0.932×10^{-3}	0.942×10^{-3}	
32	0.717×10^{-3}	1.088×10^{-3}	
33	0.751×10^{-3}	0.702×10^{-3}	
34	0.863×10^{-3}	0.968×10^{-3}	
35	0.943×10^{-3}	0.965×10^{-3}	
36	0.770×10^{-3}	0.859×10^{-3}	
37	0.809×10^{-3}		

account as a total and analyzed. And among them, 26 patients in IFNα-treated group had their lymphocyte subset retested, and further analysis showed that the level of NhTERT mRNA was much more significantly associated with the count of CD3$^+$ T lymphocytes ($r = .572$, $P = .003$, $n = 26$) and so was with the ratio of CD4$^+$/CD8$^+$ ($r = .527$, $P = .008$, $n = 26$). In addition, there were 25 patients with detectable serum HBV DNA in IFNα treated group (≥ 2.7 log copies/mL based on the lowest limit of the RT-PCR kit), and analysis showed that
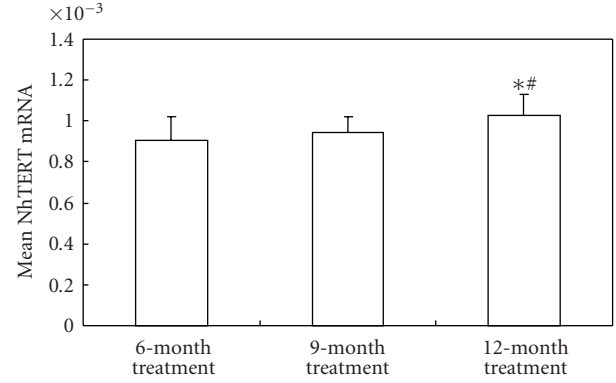
$*P = .014$, compared with 6-month treatment
$^{\#}P = .027$, compared with 9-month treatment

Comparison of Mean NhTERT mRNA levels in groups with different duration of IFNα treatment.

	No.	Mean NhTERT mRNA level ($\times 10^{-3}$)	t- value	P- value
6-month treatment	11	0.904 ± 0.117		
9-month treatment	14	0.943 ± 0.077	1.016	.320*
12-month treatment	12	1.027 ± 0.105	2.676	.014**
			2.354	.027$^{\#}$

*compared with 6-month treatment; **compared with 6-month treatment, and $^{\#}$compared with 9-month treatment.

FIGURE 2: Mean NhTERT mRNA levels in groups with different duration of IFNα treatment.

NhTERT mRNA level was not significantly correlated with HBV DNA load whether in CHB control group ($r = .230$, $P = .176$, $n = 36$) or in IFNα treated group ($r = -.209$, $P = .316$, $n = 25$).

3.5. Relationship between NhTERT mRNA and Antiviral Responses. The patients in IFNα-treated group were further divided into three subgroups in terms of their biochemical, virological, and immunological responses to IFNα therapy. We found that the mean NhTERT mRNA level was significantly higher in HBeAg loss subgroup compared with HBeAg positive subgroup ($P = .015$), and especially, the level in HBeAg loss subgroup was comparable to that in healthy control group ($P = .479$). The mean NhTERT mRNA level was higher in HBV DNA load ≤ 3 log subgroup compared with HBV DNA load >3 log subgroup but did not reach statistical significance ($P = .059$). There was no significant difference between ALT normal and abnormal subgroups ($P = .249$, Table 3).

4. Discussion

Lymphocytes are indispensable components for the host immune response, and the enough number of the cells is the basic precondition to maintain the normal immune function. During the typical immune reaction, the proliferation of lymphocytes increases 10^5-10^6 fold compared with

TABLE 3: Comparison of mean NhTERT mRNA levels between IFNα-treated subgroups.

	ALT(IU/L)		HBV DNA (log copies/mL)		HBeAg status*	
	<40	≥40	<3 log	≥3 log	e-Ag (+)	e-Ag (−)
No.	23	14	18	19	17	14
Mean NhTERT	0.967	1.007	1.011	0.950	0.910	1.010
mRNA level ($\times 10^{-3}$)	±0.105	±0.095	±0.084	±0.105	±0.111	±0.100
t-value	1.172		1.955		2.595	
P-value	.249		.059		.015	

Note. *subgroup: 31 cases of HBeAg positive CHB; e-Ag (+): without HBeAg loss; (−): with HBeAg loss.

the resting state [14]. The cellular replicative life span in normal somatic cells is finite. However, lymphocytes are the rare somatic cells to obtain the relatively enduring ability to divide, which may be, to some extent, associated with lymphocytic telomere length, because the cell replicative capacity depends largely on their telomere length. The length of human telomeric repeats is 5–15 kb [15, 16] and shortens gradually with cell division. When telomeres shorten to a certain limit, cells cannot replicate anymore and chromosomal instability and cell senescence will ensue [17]. The shortening telomere length of lymphocytes is a sign of immune senescence [18]. Telomerase can synthesize the repeat sequence TTAGGG at the telomeric ends and which can resist the shortening of telomere length; so the lymphocytes with higher telomerase activity should have more durable ability for cell division and proliferation. The researches have confirmed that the antigen-specific T lymphocytes can maintain telomere length so long as telomerase activity is high [19, 20]. In the study of HIV-infected patients, the expression of hTERT mRNA in CD4+ T lymphocytes was found significantly decreased [21, 22], which could lead to a reduced telomerase activity and shortened cell life expectancy and thus result in a limited proliferation of CD4+ T cell clones. That the lymphocytes possess a lower hTERT mRNA level may also be responsible for the decreasing number of CD4+ T lymphocytes besides the HIV direct injury to the cells. Therefore, based on the performed studies, we can find that a certain relationship exists between the telomerase activity and the immune cells.

In our study, we quantitatively detected hTERT mRNA in PBMCs from CHB patients and healthy controls. However, it was not suitable to directly compare the hTERT mRNA level among the subjects mainly because of the two reasons: (1) the count of PBMCs was not absolutely equal for each sample; (2) the RNA concentration was difficult to keep equal in the management of cellular RNA extraction for each sample. Therefore, in order to reasonably analyze the data, β-actin, a house-keeping gene which expresses invariably in all tissues and cells, was used as internal control and its mRNA was also quantitatively detected. So a relative comparison value, NhTERT mRNA, was obtained, and which reflects a relative level of hTERT expression as one copy of β-actin mRNA is expressed in PBMCs. The relative value should be stable for a certain individual and could be analyzed reasonably among the subjects. Of course, it would be much better to directly analyze hTERT mRNA if the count of isolated lymphocytes

and the concentration of extracted RNA could be obtained equally for each subject. From our data, we found that the expression of NhTERT mRNA in PBMCs of CHB patients presented significantly positive correlation with both the count of CD3+ T lymphocytes and the ratio of CD4+/CD8+, and moreover, the correlation extent seemed to be more remarkable for these indices in IFNα-treated group. NhTERT mRNA level did not show the significant correlation with the age of subjects, patient's ALT level, and the load of HBV DNA, respectively. The mean level of NhTERT mRNA was also not significantly different among the different HBV genotype groups. The results showed that the lymphocytic telomerase activity was closely associated with the number of lymphocytes and the immune function in CHB patients.

In vitro studies, it had been found that the telomerase activity significantly increased when the peripheral blood T lymphocytes were stimulated with mitogens, such as phytohemagglutinin (PHA) or phorbol ester (PMA), anti-CD3 antibody, interleukin-2 (IL-2), and other cytokines, but significantly decreased telomerase activity could be caused by use of hydrocortisone stimulating PBMCs [6, 23–25], indicating that immune regulators can exert different impacts on the expression of telomerase activity in lymphocytes. Interferon is an important immunomodulator for upregulating host immunity and thus becomes one of the important antiviral drugs approved for the treatment of CHB across the world [26]. In this study, we found that the mean NhTERT mRNA level was significantly lower in each CHB patient group than in healthy control group, which was in agreement with the result studied by Satra et al. [12]. The mean level of NhTERT mRNA in IFNα-treated group was remarkably higher than that in CHB control group, and more interestingly, a higher mean level of NhTERT mRNA was shown in the patients with longer duration of IFNα treatment. According to the stratified analysis of biochemical, virological, and immunological responses in IFNα-treated group, we found that the mean level of NhTERT mRNA in HBeAg loss subgroup was significantly higher compared with the subgroup without, and furthermore, the level in HBeAg loss subgroup was comparable to that in healthy control group; an increased level of NhTERT mRNA could be seen in HBV DNA undetectable subgroup but it did not reach to the statistical significance as compared with HBV DNA detectable subgroup; the mean levels were not of significant difference between ALT normal and abnormal subgroups. The results demonstrated that a lower telomerase activity was

expressed in PBMCs of CHB patients, and IFNα treatment could obviously upregulate the expression of hTERT mRNA. In view of the multiple cell components existed in PBMCs, therefore we will further investigate whether the change of hTERT expression is derived from the B cells or from the T cells or both, and furthermore, it is also very important to know whether the change is involved in the naïve cells or memory cells, and so forth.

In addition, we can find that there exists a close relationship between the expression of hTERT mRNA in PBMCs and IFNα treatment in CHB patients, but the exact mechanism of IFNα treatment leading to an increased hTERT mRNA expression is not yet clear. In some researches with tumor cell lines, interferon could both inhibit the expression of hTERT mRNA in leukemia cells or melanoma cells and increase its expression in certain tumor cell lines [27–29]. Recently, Hrdlickova et al. [30] conducted a research on the immune cells, and they found that interferon regulatory factor 4 and 8 correlated with the activation of telomerase promoter in macrophage cell lines, which could increase the transcription of macrophage telomerase and upregulate its activity. In our study, it is not clear whether there is a similar mechanism involved in the increased expression of hTERT mRNA in PBMCs during IFNα therapy. However, no matter what the mechanism for the increase of hTERT expression in PBMCs is, we think that the mechanism to upregulate the immune function in CHB patients treated with IFNα should at least include the role played by the increased activity of lymphocytic telomerase besides the effects of immune cells and cytokines known previously [31], because lymphocytes with higher telomerase activity attributed to IFNα treatment can resist the telomeric erosion, and thus lymphocytes will get a longer life span and more durable ability for cell division, which is the basic precondition for CHB patients to acquire a normally immune response to HBV infection.

In conclusion, we found that the telomerase activity in PBMCs was closely correlated with the total lymphocyte number and the immune function in CHB patients. The expression of telomerase activity in PBMCs of patients with chronic HBV infection was significantly lower than that in healthy individuals, and IFNα treatment could, at least in part, elevate the reduced telomerase activity in PBMCs, particularly in those who achieved HBeAg loss. Therefore, the increased telomerase activity in PBMCs of CHB patients due to IFNα therapy could extend the lymphocyte life span, maintain the durable cell division, and promote the immune function, which may be involved in a novel mechanism for IFNα antiviral therapy.

Acknowledgments

References

[1] Y. S. Cong, W. E. Wright, and J. W. Shay, "Human telomerase and its regulation," *Microbiology and Molecular Biology Reviews*, vol. 66, no. 3, pp. 407–425, 2002.

[2] K. Dubrana, S. Perrod, and S. M. Gasser, "Turning telomeres off and on," *Current Opinion in Cell Biology*, vol. 13, no. 3, pp. 281–289, 2001.

[3] P. Yan, J. M. Coindre, J. Benhattar, F. T. Bosman, and L. Guillou, "Telomerase activity and human telomerase reverse transcriptase mRNA expression in soft tissue tumors: correlation with grade, histology, and proliferative activity," *Cancer Research*, vol. 59, no. 13, pp. 3166–3170, 1999.

[4] M. P. Granger, W. E. Wright, and J. W. Shay, "Telomerase in cancer and aging," *Critical Reviews in Oncology/Hematology*, vol. 41, no. 1, pp. 29–40, 2002.

[5] L. Harrington, W. Zhou, T. McPhail et al., "Human telomerase contains evolutionarily conserved catalytic and structural subunits," *Genes and Development*, vol. 11, no. 23, pp. 3109–3115, 1997.

[6] Y. Matsumura-Arioka, K. Ohtani, T. Hara, R. Iwanaga, and M. Nakamura, "Identification of two distinct elements mediating activation of telomerase (hTERT) gene expression in association with cell growth in human T cells," *International Immunology*, vol. 17, no. 2, pp. 207–215, 2005.

[7] E. Mizukoshi, Y. Nakamoto, Y. Marukawa et al., "Cytotoxic T cell responses to human telomerase reverse transcriptase in patients with hepatocellular carcinoma," *Hepatology*, vol. 43, no. 6, pp. 1284–1294, 2006.

[8] X. X. Yao, L. Yin, and Z. C. Sun, "The expression of hTERT mRNA and cellular immunity in gastric cancer and precancerosis," *World Journal of Gastroenterology*, vol. 8, no. 4, pp. 586–590, 2002.

[9] R. B. Effros, "Telomerase induction in T cells: a cure for aging and disease?" *Experimental Gerontology*, vol. 42, no. 5, pp. 416–420, 2007.

[10] N. P. Weng, K. S. Hathcock, and R. J. Hodes, "Regulation of telomere length review and telomerase in T and B cells: a mechanism for maintaining replicative potential," *Immunity*, vol. 9, no. 2, pp. 151–157, 1998.

[11] R. Reynoso, L. Minces, H. Salomon, and J. Quarleri, "HIV-1 infection downregulates nuclear telomerase activity on lymphoblastoic cells without affecting the enzymatic components at the transcriptional level," *AIDS Research and Human Retroviruses*, vol. 22, no. 5, pp. 425–429, 2006.

[12] M. Satra, G. N. Dalekos, P. Kollia, N. Vamvakopoulos, and A. Tsezou, "Telomerase reverse transcriptase mRNA expression in peripheral lymphocytes of patients with chronic HBV and HCV infections," *Journal of Viral Hepatitis*, vol. 12, no. 5, pp. 488–493, 2005.

[13] Chinese Society of Hepatology, Chinese Medical Association, and Chinese Society of Infectious Diseases, Chinese Medical Association, "The guidelines of prevention and treatment for chronic hepatitis B," *Zhonghua Gan Zang Bing Za Zhi*, vol. 13, no. 12, pp. 881–891, 2005.

[14] J. Zhang, I. C. M. MacLennan, Y. J. Liu, and P. J. L. Lane, "Is rapid proliferation in B centroblasts linked to somatic mutation in memory B cell clones?" *Immunology Letters*, vol. 18, no. 4, pp. 297–300, 1988.

[15] R. C. Allsopp, H. Vaziri, C. Patterson et al., "Telomere length predicts replicative capacity of human fibroblasts," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 21, pp. 10114–10118, 1992.

[16] T. De Lange, L. Shiue, R. M. Myers et al., "Structure and variability of human chromosome ends," *Molecular and Cellular Biology*, vol. 10, no. 2, pp. 518–527, 1990.

[17] R. J. Hodes, K. S. Hathcock, and N. P. Weng, "Telomeres in T and B cells," *Nature Reviews Immunology*, vol. 2, no. 9, pp. 699–706, 2002.

[18] A. Satyanarayana, S. U. Wiemann, J. Buer et al., "Telomere shortening impairs organ regeneration by inhibiting cell cycle re-entry of a subpopulation of cells," *EMBO Journal*, vol. 22, no. 15, pp. 4003–4013, 2003.

[19] H. F. Valenzuela and R. B. Effros, "Divergent telomerase and CD28 expression patterns in human CD4 and CD8 T cells following repeated encounters with the same antigenic stimulus," *Clinical Immunology*, vol. 105, no. 2, pp. 117–125, 2002.

[20] A. N. Akbar and M. Vukmanovic-Stejic, "Telomerase in T lymphocytes? Use it and lose it?" *Journal of Immunology*, vol. 178, no. 11, pp. 6689–6694, 2007.

[21] O. Franzese, R. Adamo, M. Pollicita et al., "Telomerase activity, hTERT expression, and phosphorylation are downregulated in CD4+ T lymphocytes infected with human immunodeficiency virus type 1 (HIV-1)," *Journal of Medical Virology*, vol. 79, no. 5, pp. 639–646, 2007.

[22] P. Bostik, G. T. Brice, K. P. Greenberg et al., "Inverse correlation of telomerase activity/proliferation CD4+ T lymphocytes and disease progression in simian immunodeficiency virus—infected nonhuman primates," *Journal of Acquired Immune Deficiency Syndromes*, vol. 24, no. 2, pp. 89–99, 2000.

[23] B. A. Kosciolek and P. T. Rowley, "Telomere-related components are coordinately synthesized during human T-lymphocyte activation," *Leukemia Research*, vol. 23, no. 12, pp. 1097–1103, 1999.

[24] Y. Haruta, K. Hiyama, S. Ishioka, S. Hozawa, H. Maeda, and M. Yamakido, "Activation of telomerase is induced by a natural antigen in allergen-specific memory T lymphocytes in bronchial asthma," *Biochemical and Biophysical Research Communications*, vol. 259, no. 3, pp. 617–623, 1999.

[25] J. Choi, S. R. Fauce, and R. B. Effros, "Reduced telomerase activity in human T lymphocytes exposed to cortisol," *Brain, Behavior, and Immunity*, vol. 22, no. 4, pp. 600–605, 2008.

[26] M. Morgan and E. B. Keeffe, "Diagnosis and treatment of chronic hepatitis B: 2009 update," *Minerva Gastroenterologica e Dietologica*, vol. 55, no. 1, pp. 5–22, 2009.

[27] D. Xu, S. Erickson, M. Szeps et al., "Interferon α down-regulates telomerase reverse transcriptase and telomerase activity in human malignant and nonmalignant hematopoietic cells," *Blood*, vol. 96, no. 13, pp. 4313–4318, 2000.

[28] C. Miracco, E. Maellaro, L. Pacenti et al., "Evaluation of MDR1, LRP, MRP, and topoisomerase IIalpha gene mRNA transcripts before and after interferon-alpha, and correlation with the mRNA expression level of the telomerase subunits hTERT and TEP1 in five unselected human melanoma cell lines," *International Journal of Oncology*, vol. 23, no. 1, pp. 213–220, 2003.

[29] E. Maellaro, L. Pacenti, B. Del Bello et al., "Different effects of interferon-α on melanoma cell lines: a study on telomerase reverse transcriptase, telomerase activity and apoptosis," *British Journal of Dermatology*, vol. 148, no. 6, pp. 1115–1124, 2003.

[30] R. Hrdličková, J. Nehyba, and H. R. Bose, "Regulation of telomerase activity by interferon regulatory factors 4 and 8 in immune cells," *Molecular and Cellular Biology*, vol. 29, no. 3, pp. 929–941, 2009.

[31] E. Loggi, A. Gramenzi, M. Margotti et al., "In vitro effect of thymosin-alpha1 and interferon-alpha on Th1 and Th2 cytokine synthesis in patients with eAg-negative chronic hepatitis B," *Journal of Viral Hepatitis*, vol. 15, no. 6, pp. 442–448, 2008.