Key Immune Cell Cytokines Affects the Telomere Activity of Cord Blood Cells *In vitro*

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Abstract

Purpose: Telomere is a nucleoprotein complex at the end of eukaryotic chromosomes and its length is regulated by telomerase. The number of DNA repeat sequence $(TTAGGG)_n$ is reduced with each cell division in differentiated cells. The aim of this study was to evaluate the effect of SCF (Stem Cell Factor), Flt3 (Fms- Like tyrosine kinase-3), Interleukin-2, 7 and 15 on telomere length and hTERT gene expression in mononuclear and umbilical cord blood stem cells (CD34+ cells) during development to lymphoid cells.

Methods: The mononuclear cells were isolated from umbilical cord blood by Ficoll-Paque density gradient. Then cells were cultured for 21 days in the presence of different cytokines. Telomere length and hTERT gene expression were evaluated in freshly isolated cells, 7, 14 and 21 days of culture by real-time PCR. The same condition had been done for CD34+ cells but telomere length and hTERT gene expression were measured at initial and day 21 of the experiment.

Results: Highest hTERT gene expression and maximum telomere length were measured at day14 of MNCs in the presence of IL-7 and IL-15. Also, there was a significant correlation between telomere length and telomerase gene expression in MNCs at 14 days in a combination of IL-7 and IL-15 (r = 0.998, p =0.04). In contrast, IL-2 showed no distinct effect on telomere length and hTERT gene expression in cells.

Conclusion: Taken together, IL-7 and IL-15 increased telomere length and hTERT gene expression at 14 day of the experiment. In conclusion, it seems likely that cells maintain naïve phenotype due to prolonged exposure of IL-7 and IL-15.

Introduction

Telomeres are nucleoprotein structures at the end of eukaryotic chromosomes. As it is proven, DNA hexamer $(TTAGGG)_n$ repeat have an important role in chromosomes stability.[1,2] In differentiated cells, the number of DNA hexamer repeats decreases with each cell division.[3-5]

Telomerase is encoded by the telomerase reverse transcriptase (TERT) gene and it associated with a telomerase RNA component (TERC) as RNA template. This enzyme adds telomeric repeats to the chromosome ends and prevents telomere shortening in germ line cells and the majority of tumor cells.[6,7] Therefore, telomere length and expression level of telomerase positively correlate with the immortality of cancer cells, germ-line cells and embryonic stem cells.[8-11]

In many previous studies has been demonstrated that telomere lengths change in lymphocytes with activation and differentiation of T and B cells. In parallel telomerase is expressed in normal lymphocytes, and this expression is regulated in the time of development and activation. These findings indicate that the maintenance of telomere length may be important to regulate T- and B-cell responses.[12,13] There is a strong correlation between telomere length and replicative capacity in all normal cells. This correlation extends in T cells to differences in telomere length and replicative capacity in subsets such as naive and memory CD4+ cells. Furthermore, telomerase expression is highly regulated during development and activation of both T and B cells.[14]

IL-2, IL-7 and IL-15 are critical cytokines, regulating in hematopoiesis and proliferation, self-renewal, differentiation and senescence of HSCs (Hematopoietic stem cells).[15,16] Interleukin- 2 (IL-2) supports hematopoiesis, T cell proliferation (both CD4+ and CD8+) and cellular metabolism.[17-20] As well as the

expansion of activated T lymphocytes (T cells) and differentiation of B cells.[21] In addition, previous studies revealed that in T-cells recently exposed to IL-2 apoptosis is more likely induced upon antigen-dependent T-cell receptor stimulation.[22] IL-2 along with Interleukin-15 (IL-15) are growth factors for natural killer cells.[23] It is shown that IL- 7 influences the expansion of B cells, promotes T cell differentiation and has an important role in natural killer cell development.[24] Additionally, Interleukin-15 (IL-15) promotes expansion, proliferation and homeostasis of T cells.[25]

We have previously shown that B and T cell expansion increased in cord blood mononuclear cells using IL-2 and IL-7.[26] In another study, we illustrated that IL-2 and IL-15 are important for the expansion of NKP46 positive cells (NK cells) in cord blood cells in vitro.[27]

Cord blood is a major source of CD34 positive cells used HSC-transplantation in leukemia treatment or cancer immunotherapy. So far, it remains unclear if cytokines can interfere with telomerase dynamic in umbilical cord blood cells.

The aim of this study was to evaluate the influence of IL-2, IL-7 and IL-15 on the telomere length and hTERT gene expression on mononuclear and CD34$^+$ umbilical cord blood stem cells during development to the lymphoid cell.

Materials and Methods

Mononuclear cord blood isolation and CD34$^+$ cells enrichment

Umbilical cord blood sample were obtained from full-term normal deliveries in Tabriz Alzahra hospital, Iran. The collected cord blood was diluted 1:2 with phosphate buffered saline (PBS) plus 10% fetal bovine serum (FBS). Mononuclear cells (MNCs) were isolated by Ficoll-Hypaque (GE Healthcare, Piscatta, NJ, USA) gradient centrifugation at 400×g for 25 min at 4° C. Subsequently MNCs were collected and washed twice in PBS plus 5% FBS. The isolated MNCs were co-incubated with 100 μl of CD34$^+$ micro beads (Miltenyi Biotec, Germany Cat no: 130100453) for 30 minutes. Thereafter resuspended cells were passed through one LS MACS column (Miltenyi Biotec, Germany). Thereafter enriched CD34$^+$ cells were retrieved by flushing the column. For purity assessment of CD34$^+$ cells, flow cytometry was performed by FACSCalibur (BD Bioscience) and the output data were processed with Flow software version X.0.7.

Culture condition

MNCs and isolated CD34$^+$ cells were seeded in 96-well flat bottom cell culture plates at the density of $5×10^5$ cells per well. For culturing RPMI 1640 supplemented with 20% (v/v) FBS and 1% (v/v) penicillin/streptomycin was used. Different combinations of cytokines (Pepro Tech, London, UK) were added to the culture medium in final concentrations of 80 ng/ ml and cell cultures were maintained for 21 days. SCF and Flt3 were same in all groups. Cells were harvested and analyzed at day 7, 14 and 21. All cytokines used in this study including SCF, FLT3, IL-2, IL-7 and IL-15 were purchased from Peprotech.

Flow cytometry

Monoclonal antibodies were CD3 (UCHT1; R&D) for T cells, CD20 (PE; clone 2H7; BD Biosciences) for B cells and Anti-NKp46-PE (BD,biosience) for NK cells. Flow cytometry was performed at 14 day of culture period time. Shortly, the cells were incubated with these antibodies in each group (for 20 min at 4ºC). We used of Propidium iodide (1.0 mg/mL; Invitrogen) to eliminate of dead cells. The flow cytometry analysis of the cell suspensions was performed on a BD FACSCalibur analyser (BD Biosciences). Between 10000 and 30000 events were collected and analysed by the flow cytometry software Perttu Terho (version: 2.5.1., Cyflogic, Finland).

RNA and DNA extraction

Total RNA was extracted from harvested MNCs and CD34$^+$ cells at different time points using Thermo Scientific Gene JET RNA Purification Kit (K0731) according to the manufacturer's instruction. Extracted RNA was dissolved in nuclease-free water. For integrity assessment of extracted RNA we use of %1.5 agarose gel electrophoresis and for purity assessment we use of spectrophotometry. DNA was extracted using the pure link genomic DNA mini kit (Invitrogen, USA) according to the manufacturer's instruction. Briefly, the cells were collected and digested with Proteinase K (Invitrogen, USA) at 55 °C. Any residual RNA was removed by incubation with RNase A (Invitrogen, USA) for 2min. After digestion, 200 μl ethanol (96~100%) was added, mixed thoroughly by pulse vortexing for 30 sec, and transferred carefully to a DNA binding pure link spin column. Finally, impurities were removed by washing buffer. The genomic DNA was then eluted in the low salt elution buffer. For integrity assessment we use of %1 agarose gel electrophoresis and purity assessment was analysed by spectrophotometry.

c-DNA synthesis and Real-time PCR

Reverse transcription was carried out using the RevertAid^{TM} first strand cDNA synthesis kit (K1622; Fermentas, Germany). Two microgram RNA was used for the first-strand cDNA synthesis in a total volume of 20 μL according to the manufacturer's guidelines. Briefly, the reaction tubes were put at 65°C for 10 min, 42°C for 60 min preceded with 70°C for 10 min and followed at 4°C for 5 min. For every reaction set, one RNA sample was prepared without RevertAid^{TM} M-MuLV reverse transcriptase (RT reaction) to provide a negative control in the subsequent PCRs.

All PCRs were performed using the Corbett Rotor-Gene™ 6000 HRM (Corbett Research, Australia) in a total volume of 20 μL containing Power SYBR Green master mix (2x) (TaKaRa Ex Taq HS, Japan), Primer (0.4 μM), cDNA (20 ng/μl) plus nuclease free water. The

mRNA-specific primers were designed using Oligo 7 software (v. 7.52, Molecular Biology Insights, Inc, USA). The sequences are listed in Table 1 and 2. Both *β-actin* and *hTERT* amplification were done in triplicates for each sample. *β-actin* was selected as an endogenous housekeeping gene. Forty-five thermal cycles were performed in the following order: 2 min at 94ºC, 40 cycles, 94ºC for 15 sec and 63ºC for 1 min. PCR data were analyzed using Rotor-Gene 6000 Software (version: 1.7) to determine CT values. Delta CT values were calculated in relation to *β-actin* CT values by the $2^{-\Delta\Delta CT}$ method, in which ΔCt represents the difference between the CT value of target genes and the CT value of *β-actin*.

Table 1. Primers for quantitative Real-time RT-PCR

No.	Gene	Primer pair sequence (5'-3')	Product length (bp)
NM_001193376.1	TERT	CCGCCTGAGCTGTACTTTGT CAGGTGAGCCACGAACTGT	234
NM_001101.3	β-actin	AAACTGGAACGGTGAAGGTG TATAGAGAAGTGGGGTGGCT	174

Table 2. Oligomers used for aTL assay

Oligomer name	Oligomer sequence (5'-3')	Amplicon size (bp)
Telomere standard	(TTAGGG)$_{14}$	84
36B4 standard	5'CAGCAAGTGGGAAGGTGTAATCCGTCTCCACAGACAAGGCCAGGA CTCGTTTGTACCCGTTGATGATAGAATGGG-3'	75
Telo	Fwd:CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT Rev:GGCTTGCCTTACCCTTACCCTTACCCTTACCCT	>76
36B4	Fwd:CAGCAAGTGGGAAGGTGTAATCC Rev:CCCATTCTATCATCAACGGGTACAA	75

Standard curves and associated calculations for aTL

A standard curve was obtained from dilution series of known quantities of a synthesized 84 mer oligonucleotide (84 bp in length) containing only TTAGGG repeated 14 times. The number of repeats in each standard is calculated as previously described by O'Callaghan.[2] For generating a standard curve the serial dilutions of TEL STD A (10^{-1} [1.18×10^8] through to 10^{-6} [1.18×10^3] dilution) is performed. Plasmid DNA (pet 28a) was added to each standard to maintain a constant 20 ng of total DNA per reaction tube (Table 3).

Table 3. Amounts of calculation for aTL

Oligomer	Molecular weight (MW)	Weight of telomere standard and 36B4 (g)	Number copies of 36B4	Number molecules of oligomer in TEL STD A	Amount of telomere sequence in TEL STD A (kbp)	SCG STD A
(TTAGGG)14	26667.2.	$2.6667 \times 10^4/6.02 \times 10^{23} = 0.44 \times 10^{-19}$	-	$60 \times 10^{-12}/0.44 \times 10^{-19} = 1.36 \times 10^9$	$1.36 \times 10^9 \times 84 = 1.18 \times 10^8$	-
synthesized 36B4 oligomer standard	23268.1	$2.32681 \times 10^4/6.02 \times 10^{23} = 0.38 \times 10^{-19}$	$200 \times 10^{-12}/0.44 \times 10^{-19} = 5.26 \times 10^9$	-	-	2.63×10^9

For the single copy gene (SCG) standard curve, we routinely used 36B4, which encodes the acidic ribosomal phosphoprotein P0. Although telomeric DNA sequence is consistent in mammals, the SCG will be different, thus, an SCG standard curve and amplicon must be generated for each target species. SCG amplification is crucial for the accuracy and reliability of the results generated in the aTL assay.

For generating a standard curve the serial dilutions of SCG STD a (10^{-1} through to 10^{-6} dilution) is performed (Figure 1). As same as telomere standard, plasmid DNA (pet 28a) is added to each standard to maintain a constant 20 ng of total DNA per reaction tube.

Statistical analysis

In this study data were studied by one-way ANOVA followed by the Tukey test. For graph we used of Prism software (GraphPad Software, Inc., San Diego, CA version; 6). Values were measured statistically significant at $P < 0.05$.

Results

The IL-2, IL-7 and IL-15 can stimulate T, B and NK cells expansion in cord blood mononuclear cells

5×10^5 cells were cultured in presence of different combination of cytokines including IL-2, IL-7 and IL-15 for 14 days. Harvested cells evaluated at 14 day by flow

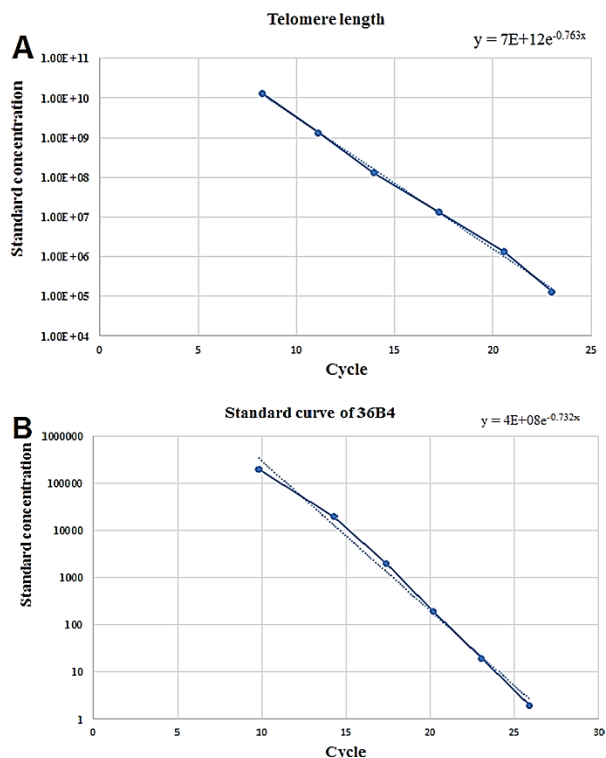cytometry using CD 20 for B cells, CD3 for T cells and NKP46 for NK cells.

Figure 1. Standard curve used to calculate absolute telomere length.**(A)** Graph shows standard curve for calculating length of telomere sequence per reaction tube. X-axis represents number of cycle and Y-axis show the standards concentration.**(B)** Graph shows standard curve for calculating genome copies using 36B4 copy number. X-axis represents number of cycle and Y-axis show standard concentrations per each reaction.

As shown in Figure 2 the IL-2 are involved in expansion of T (94%), B (96%) and NK cells(38%). However IL-7 increased T cell expansion (92%) as well as B cells (97%), but not significantly increased NK cells. Our data were shown that IL-15 can increase the expansion of NK cells (28%), but it was not important for B cells. The Figure 2 shows that IL-2 and IL-15 can stimulate expansion of mononuclear cells toward T and NK cells and IL-7 was important for B cells.
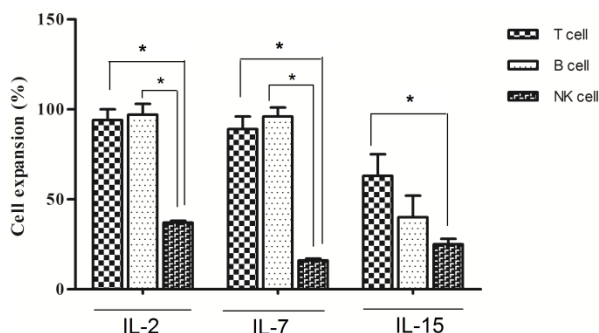

Figure 2. Cord blood mononuclear cell derived T, B and NK cells in presence of IL-2, IL-7 and IL-15 at day 14 in vitro. Harvested cells evaluated by flow cytometry and measured the percentage of B, T and NK cells. In all groups SCF and FLt3 included. All data showed mean ± SD. Differences between groups are significant at *p <0.05.

The effect of Interleukin-7 on telomere length and hTERT gene expression in cord blood mononuclear cells

To investigate the effects of IL-7 on both telomere length and hTERT gene expression, quantitative real-time PCR was carried out in MNCs cultured with IL-7 for 21 days. We found that IL-7 could actively alter the dynamic of telomere length over a period of 21 days: The minimal telomere length was found in non-cultured cells (0.86 kb). The absolute telomere length was elongated in day14 (56.82 kb) in compared to day7 while it shortened (9.09 kb) in day 21 days in compared to day14 (Figure 3A). A simultaneous evaluation of mean fold of hTERT in day 7, 14 and 21 was 2.36, 3.94 and 0.97, respectively (Figure 3B). In parallel highest hTERT expression was seen at day 14 (3.94) and the significant difference was seen in days 14 and 21 ($P<0.01$).
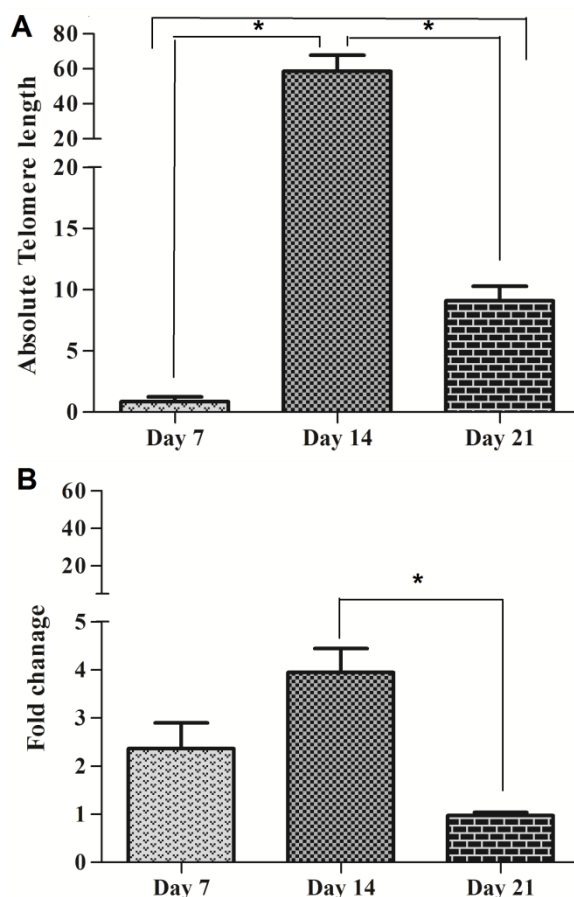

Figure 3. comparison of telomere length and hTERT gene expression folds in MNCs after treatment with 80 ng/ml of IL-7.(**A**) Telomere length (Kbp) in 7, 14 and 21 days of MNCs. (**B**) hTERT gene expression fold in MNCs in 7, 14 and 21 days. Differences between groups are significant at *p <0.05, **p <0.01, and ***p<0.001.

Association between telomere length and hTERT gene expression in cord blood mononuclear cells is an outcome of CD34+ stem cells expression using Interleukin-7 (IL-7)

We cultured 4×10^5 CD34+ enriched cord blood cells for 21 days with IL-7. Telomere length and hTERT gene

expression were measured in non-cultured and late time point of our experiment (day 21). Similar to MNCs, telomere length and hTERT gene expression were measured. The mean telomere length of CD34^{+} cells was recorded 0.12 and 0.129 kb at day 0 and 21 respectively. No significant differences were seen in telomere length at the beginning and day 21 ($P>0.05$) (Figure 4A). Accordingly, the mean hTERT gene expression fold was 0.14 in day 0 and reached 9.79 at day 21 while no significant differences were observed in gene expression levels of hTERT between day 0 and 21 ($P= 0.06$) (Figure 4B).
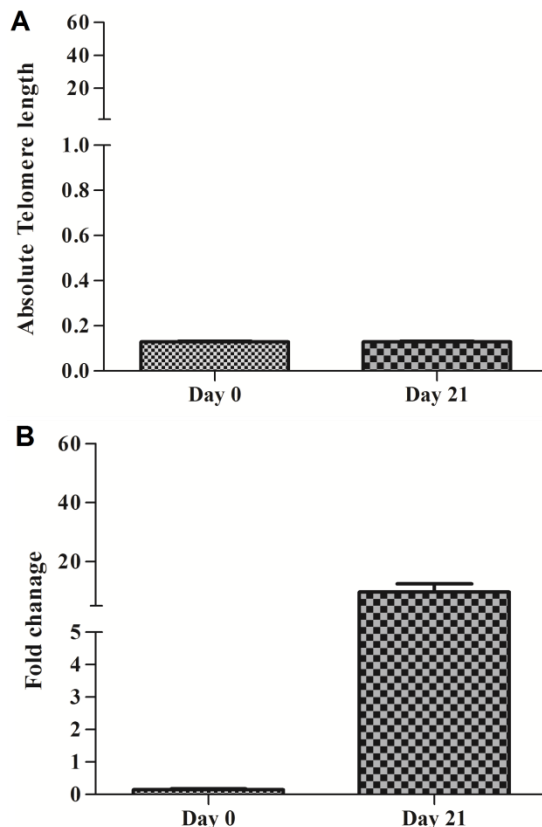

Figure 4. Relationship between of telomere length and hTERT gene expression folds in CD34^{+} cells in using 80 ng/μl of IL- 7. (**A**) Telomere length (Kbp) of CD34^{+} cells in 0 and 21 days. (**B**) hTERT gene expression fold of CD34^{+} in 0 and 21 days. Differences between groups are significant at *p <0.05, **p <0.01, and ***p<0.001.

Association between Immune cell key cytokines with Telomere length and hTERT gene expression in cord blood MNCs

To evaluate telomere length and hTERT gene expression, quantitative real-time PCR was performed at day 7, 14 and 21 for harvested cells.

Average telomere length of MNCs after 14 days of co-culture with IL- 2, IL- 7 and IL- 15 was 7.42 kb, 58.62 kb and 22.52 kb. Interestingly, the highest length of telomere was observed after co-culture with IL- 7 (Figure 5A). Also, the mean hTERT gene expressions measured in cultured cells with IL-2, IL-7 and IL-15 and were in order 1.35, 17.34, 3.94 and 48.54. The

significant differences were indicated for cultured with IL- 2 (P< 0.01), IL-7 (P<0.05) and IL-15 (P<0.001) (Figure 5B). The highest hTERT gene expression fold was seen in cultured with IL-15.
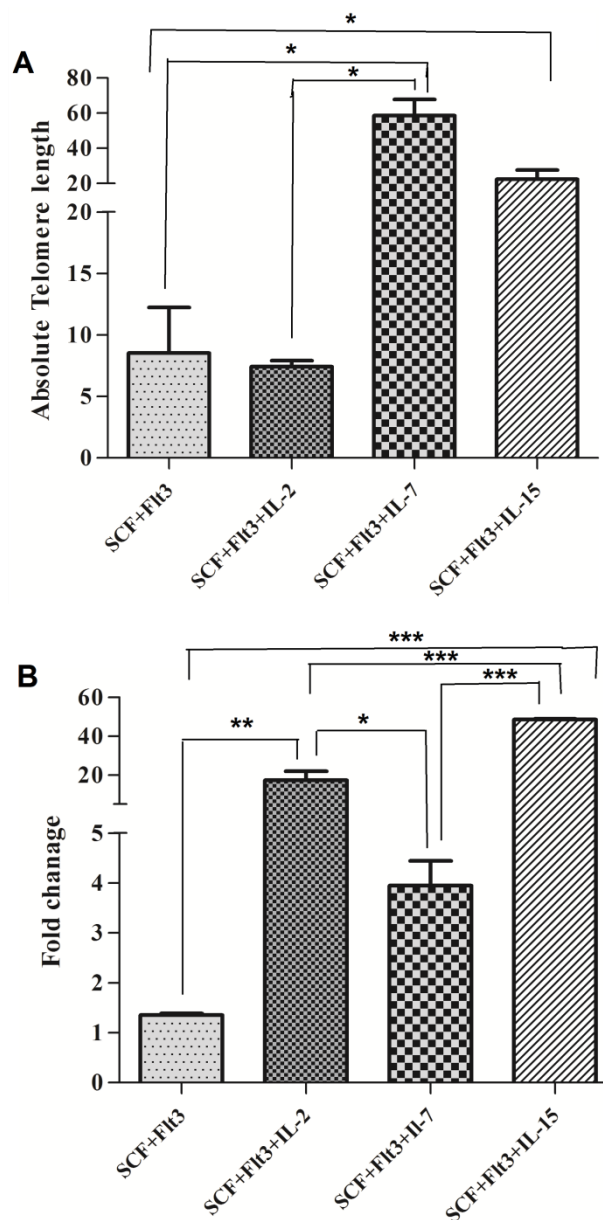

Figure 5. Comparisons of telomere length and hTERT gene expression fold in different groups at 14th day of MNCs (using 80ng/μl of each cytokines). (**A**) telomere length (Kbp) in SCF+FLT3, SCF+FLT3+IL-2, SCF+FLT3+IL-7 and SCF+FLT3+IL-15 groups at 14th day of MNCs.(**B**) hTERT gene expression fold in SCF+FLT3, SCF+FLT3+IL-2, SCF+FLT3+IL-7 and SCF+FLT3+IL-15 groups at 14th day of MNCs. Differences between groups are significant at *p <0.05, **p <0.01, and ***p<0.001.

Correlation between hTERT gene expression and cellular turnover

Telomere length and hTERT gene expression were evaluated by real-time PCR in distinct time points for cultured cells with the combination of IL-2, IL-7 and IL-15.

Telomere length at day 7, 14 and 21 accounted for 17.2 kb, 14.4 kb and 14.4 kb, respectively and there were no significant differences between groups (P>0.05) (Figure 6A). Mean hTERT gene expression was 3.26, 33.11 and 1.44 in days 7, 14 and 21 of culture respectively. Significant differences were seen at day 14 and 21 (P<0.001) (Figure 6B). Also there was correlation between telomere length and hTERT gene expression at 14 day (r = 0.998, p =0.04).
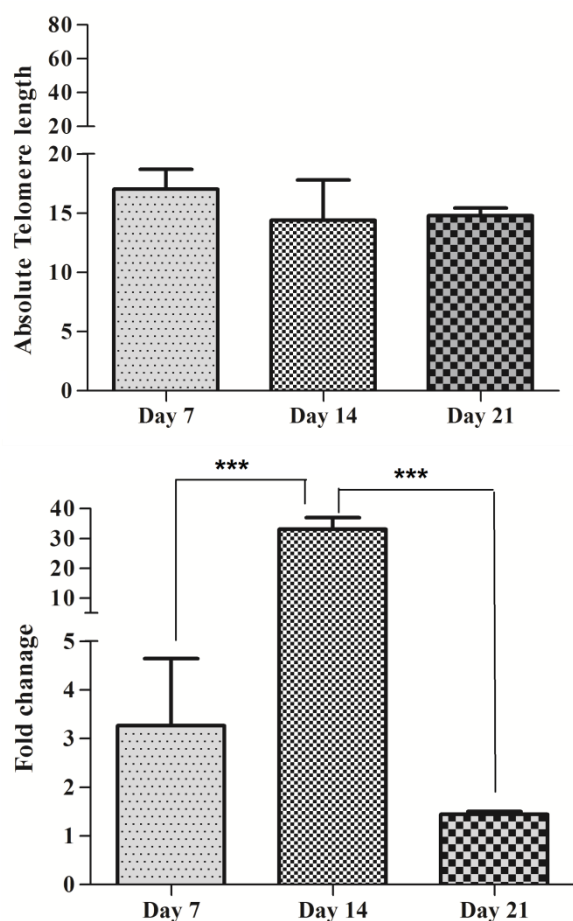

Figure 6. association between of telomere length and hTERT gene expression Fold in SCF+FLT3+IL-2+IL-7+IL-15 group of MNCs (using 80ng/μl of each cytokines). (**A**) Telomere length (Kbp) in 7, 14 and 21 days of MNCs. (**B**) hTERT gene expression fold in 7, 14 and 21 days of MNCs. Differences between groups are significant at *p <0.05, **p <0.01, and ***p<0.001.

Both Telomere length and hTERT gene expression in MNCs and CD34 positive cells are high using Interleukin-15 (IL-15)

Both MNC and CD34$^+$ cells (n= 4 ×10^5) were co_cultured with IL-15 and telomere length and hTERT gene expression were measured at different time points. Telomere length was measured in MNCs by real-time PCR. Absolute telomere length was 13.68 kb at day 7, 22.52 and 0.7 kb at day 14 and 21, respectively. The highest telomere length was seen at day 14. There was no significant difference between groups (P>0.05) (Figure 7A). hTERT gene expression fold at day 7, 14 and 21

day was 2.73, 48.54 and 1.34, respectively. Results showed that there are significant differences in hTERT gene expression between day 14 and 21(P<0.001). The highest hTERT gene expression fold was observed at day 14 (Figure 7B).

Final telomere length of CD34$^+$ accounted for 0.12 kb in non-cultured cells and 0.58 kb in cells cultured for 21 days. There was no significant difference between telomere length at noncultured cells and cells cultured for 21 days (P>0.05) (Figure 7C). hTERT gene Expression fold was 0.14 at noncultured and 0.11at day 21. There was no significant difference between hTERT expression in non-cultured and at day 21 in CD34$^+$ cells (P>0.05) (Figure 7D).

Discussion

Natural killer cell, T and B lymphocyte development are controlled by immune cell cytokines (IL-2, IL-7 and IL-15) which known as common cytokine gamma chain. Previous study demonstrated that there is a relationship between B cell development and IL-2 and IL-7.[28] Our results showed that IL-2 and IL-7 affect the expansion of T and B cells more than NK cells at 14 day of culture. NK cell development is dependent to two member of common cytokine gamma chain on the other hand IL-2 and IL-15.[29] Similar to previous study, NK cells expansion percent was higher in IL-2 and Il-15 group more than IL-7 group.

In the present study, absolute telomere length and hTERT gene expression were evaluated in cord blood-derived MNCs and CD34$^+$ cells after treatment with SCF, FLT3, IL- 2, IL- 7 and IL- 15.We found that, telomere length and hTERT expression were higher after 14 days of exposure to IL-7 and IL-15. Blackburn et al reported reduced telomerase function in proliferating cells and the population of heterogeneously sized telomeres with time shortens and the cell population undergoes telomere shortening and in experiments that telomerase enzyme was disrupted the length of telomere-shortened in cells.[30,31] In line with this statement, disturbed hTERT function resulted in telomere shortening and cellular senescence.[30,32] It was previously shown several times that the up-regulation of hTERT expression has a key role in the maintenance of telomere length and cell proliferation capacity.[33] It is commonly believed that IL-7 is required for homeostatic proliferation of naïve T cells while IL-15 stimulates maturation of memory T cells.[34] Previously, Diana et al unveiled that a long-term culture of cells in the presence of both IL-7 and IL-15 cytokines resulted in the delayed expansion of naïve CD8$^+$ T cells that cellular expansion rate peaked only after 14 days.[34] In line with our current results, previous investigations of human fibroblasts revealed that telomere shortening correlated with replicative senesces *in vitro*.[35] Here, we demonstrate that IL-7 or IL-15 treated cells could retain their naïve phenotype and telomere length through up-regulation of hTERT expression.[36] The telomere length of IL-7 treated MNCs reached its maximum level at day 14 of the

experiment which may be due to the late expansion of T cell in response to IL-7. Yu Li et al described the potency of IL-15 to induce telomerase expression during activation of the Jak3 and PI3K/ AKT signaling pathways in T lymphocytes. Particularly in memory CD8^{+}T cells.[37] On the other hand, IL-15-induced telomerase dynamic is associated with the relative stable telomere length in long-term cultured memory phenotype CD8^{+}T cells.[37] The induction of hTERT via cytokines is distinctly mediated by PI3K/Akt, or the NF-kB pathways. Among the possible mechanism suggested for NF-kB pathway, protein kinase C (PKC) has been shown to have a critical role in up-regulation of TERT expression.[38]
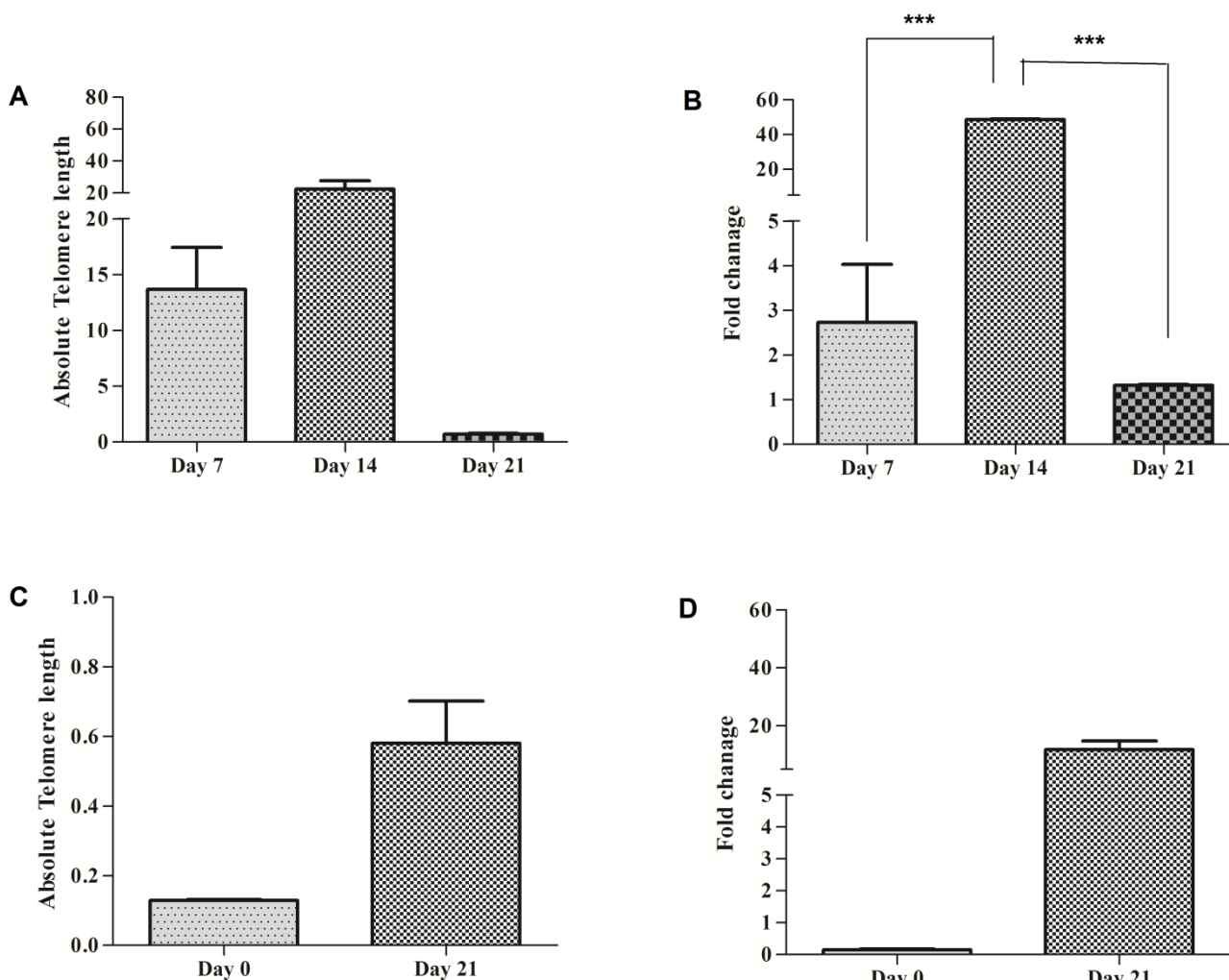

Figure 7. Comparison of telomere length and hTERT gene expression folds in MNCs and CD34^{+} in using 80 ng/ µl of IL-15. (**A**) Telomere length (Kbp) in MNCs in different days of culture period time. (**B**) hTERT gene expression fold of MNCs in different days of culture period time. (**C**) Telomere length (Kbp) of CD34^{+} cells in 0 and 21 days. (**D**) hTERT gene expression fold of CD34^{+} in 0 and 21 days. Differences between groups are significant at *p <0.05, **p <0.01, and ***p<0.001.

In CD34^{+} cells, telomere length and hTERT gene expression analysis showed that telomere length was higher at the 21th day, thereby indicating that prolonged exposure to IL-7 its effects. The CD34^{+} cord blood cells proliferated faster in response to cytokine stimulation and total cell proliferation was increased sevenfold compared to their counterpart bone marrow.[39] Study of colony-forming dynamic by sorted cord blood and bone marrow CD34^{+} cells revealed that, although the number of CD34^{+} cells in cord blood is less than bone marrow, cord blood CD34^{+} cells have a significantly higher number of colony formation.[36] This is possible that telomere length and hTERT gene expression in MNCs is an outcome of CD34^{+} cells.

In this experiment cells were treated with different cytokines including; SCF, FLT3, IL- 2, IL- 7 and IL- 15. In consequence telomere length and hTERT gene expression were altered. We showed that telomere length in combination with cytokines was constant at different time point but hTERT expression at 14th day was highest. These results can be explained by roles of cytokines in cell grows regulation of subtypes of immune cells and may be in this situation hTERT gene expression level have no effect on telomere length and telomere length controlled by alternative lenghething telomere

mechanism. Another theory may be is when cytokines used with together telomere length not affected by hTERT expression level and remodeling in chromatin structure cause to this condition. In the other manner, in this condition telomere length not regulated by hTERT level.[40,41]

Conclusion

In conclusion, this study showed that telomere length and hTERT gene expression are affected by IL-7 ad IL-15. The longest telomere length and highest hTERT gene expression were observed at 14th day of culture when cells treated with Il-7 and Il-15. But IL-2 has no distinct effect on telomere length and hTERT gene expression.

Acknowledgments

Ethical Issues

Not applicable.

Conflict of Interest

All author declared no conflict of interest.

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