Correlation between telomere maintenance and stemness of mesenchymal stem/stromal cells

Drenka Trivanović¹, Jelena Krstić¹, Aleksandra Jauković¹, Branka Popović², Ivana Okić Djordjević¹, Tamara Kukolj¹, Hristina Obradović¹, Slavko Mojsilović¹, Juan Francisco Santibanez¹, Diana Bugarski¹

¹Laboratory for Experimental Hematology and Stem Cells, Institute for Medical Research, University of Belgrade, 11129 Belgrade, Serbia
²Institute of Human Genetics, School of Dentistry, University of Belgrade, 11129 Belgrade, Serbia

Correspondence: Drenka Trivanović
E-mail: drenka.trivanovic@imi.bg.ac.rs
Received: January 10, 2016
Published online: February 16, 2016

Tissue regeneration and repair is strongly dependent on resident stem cells behavior and functionality. Telomeres and telomerase are known to be regulators of stem cell self-renewal, controlling their cell cycle, thus determining their replicative potential. Also, telomeres and telomerase are involved in regulation of differentiation progress of stem cells, although these effects are poorly understood. In previously published study, we compared relative telomere length (RTL) and hTERT mRNA expression in mesenchymal stem/stromal cells (MSCs) isolated from: peripheral blood (PB-MSCs), umbilical cord (UC-MSCs), exfoliated deciduous teeth (SHEDs), periodontal ligament (PDL-MSCs) and adipose tissue (AT-MSCs), in context of their proliferation and differentiation. We found that PB-MSCs and UC-MSCs demonstrated the highest RTL and hTERT mRNA expression, accompanied with their higher proliferation capacity and adipogenic differentiation in comparison with other MSCs. On the other hand, SHEDs, PDL-MSCs and AT-MSCs demonstrated lower RTL value and hTERT mRNA expression accompanied with higher osteogenic differentiation capacity and higher expression of pluripotency markers (Nanog, Oct-4A, Oct-4B and SOX-2) mRNA, when compared to PB-MSCs and UC-MSCs. These results highlighted the difference between MSCs isolated from various tissues with indications that telomere status might be one of the predictable factors for their stemness and functionality, prior to their potential application in cell-based therapy.

Keywords: mesenchymal stem/stromal cell; telomere, stemness; proliferation; pluripotency markers

To cite this article: Drenka Trivanović, et al. Correlation between telomere maintenance and stemness of mesenchymal stem/stromal cells. Telomere Telomerase 2016; 3: e1184. doi: 10.14800/tt.1184.

Copyright: © 2016 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

Tissue development is governed by dynamic equipoise of senescence and apoptosis of terminally differentiated cells on one side, and appropriate gradual differentiation of rare resident stem or progenitor cells in tissues on the other side. These processes require strict control of cell-cycle progression, followed by DNA replication, which are conditioned by chromosome stability provided by telomere replication and telomerase-mediated telomere extension in S phase. Due to the role in cell-cycle control and genome stability, telomere status is an important feature of stem, progenitor or precursor cells involved in maintenance of tissue homeostasis [1-2].
In short, telomeres protect DNA ends present at the termini of linear chromosome, prevent broken ends to act, by inhibiting telomere fusions, illegitimate recombination, and genomic instability, which could lead to cellular transformation [3, 4]. Telomeric DNA acts with telomere-associated proteins to form additional structures (such as G-quadruplexes and T-loops) and in some regions double-stranded DNA (the D-loop) which are responsible for the prevention of the irregular activation of checkpoints and DNA repair response at chromosome ends [15, 6].

About 50 bp of telomeric repeats are lost per generation (division), due to inability of the semiconservative DNA replication to replicate the entire telomere [7]. Telomere maintenance requires telomerase, network of telomere-associated proteins such as telosome and shelterin, and also the inhibited DNA-damage response signals that include ATM and ATR pathways, homologous recombination, and non-homologous end joining [6]. Repair response could also activate alternative mechanisms of telomere lengthening (ALT), contributing to the capacity of transformed cells to gain infinite growth capacity [5].

Telomerase is a ribonucleoprotein complex and acts as a reverse transcriptase, which compensates telomere loss, as well as loss accomplished by nucleolytic processing, by catalyzing the addition of telomeric hexanucleotide repeats onto the 3’ end of the chromosome [7]. Telomerase consists of the telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC) as the template for the telomerase extension during addition of TTAGGG repeats onto chromosome ends. Contrary to ubiquitous TERC expression, expression of TERT is highly regulated. Although TERT expression and telomerase activity are hardly detectable in somatic cells, telomerase is expressed in stem cells and highly expressed in cancer cells [6].

Telomere biology became a field that holds great promise for medicine, particularly for aging and age-related diseases, including diabetes mellitus, cardiovascular diseases, liver disorders and cancer, and their status in stem cells as compartments which contribute to tissue homeostasis obtained particular attention [8].

Importance of Telomere Maintenance for Stem Cell Properties

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and engineering-derived induced pluripotent stem cells (iPSCs), have the potential to produce any type of cells from all three basic germ layers and the capacity to self-renew and proliferate indefinitely in vitro, and therefore hold unrivaled promise for regenerative medicine. Based on previous data that demonstrated a crucial role of overexpressed TERT in the generation of iPSCs [9, 10], it is suggested that telomerase has major role in reprogramming, self-renewal and maintenance of iPSCs pluripotency [11].

Except stem cells and lymphocytes, most human somatic cells gain abolished telomerase activity after birth. The level of telomerase is low in the majority of human adult stem cells, except in cells that undergo rapid expansion, such as committed hematopoietic progenitor cells [12], activated lymphocytes [13], or keratinocytes [14, 15, 16, 17]. Tissue resident stem cells are present in almost all tissues, and therefore represent the most investigated primary stem cells. Due to their availability and abundance in various adult or birth-associated tissues they take important place in regenerative medicine approaches.

Since scarce populations of hematopoietic stem cells (HSCs) have the ability to repopulate myeloablated bone marrow, it is suggested that these cells possess extensive replicative capacity. Besides this feature of HSCs demonstrated both in laboratory and in clinical settings, it was described that their replicative capacity is limited and dependent on telomere shortening [18]. Cautions about telomere length of HSCs are related to their application in HSCs transplantation (HSCT). It is shown that cells from old donors or patients with telomerase deficiencies had limited replication ability, rendering the cell number necessary for HSCT insufficient [19]. Moreover, in patients who had received HSCT, the telomere length of engrafted stem cells is closely related to the outcomes of transplantation, thus highlighting the significance of telomere status during donor selection [20]. Therefore, some authors speculate about advantages of cord blood HSCs use, which are related to longer telomeres and higher replication ability than HSCs in bone marrow [19]. Telomere shortening in HSCs is considered to be a risk factor that contributes to the development of chromosomal instability and malignant transformation. In agreement with this hypothesis, telomere shortening in patients with dyskeratosis congenita correlates with an increased risk of hematological neoplasms [15, 21].

As already mentioned, mesenchymal stem/stromal cells (MSCs) have been isolated from many different birth-associated or adult tissues: e.g. umbilical cord, amniotic fluid, placenta, bone marrow, adipose tissue, peripheral blood, urine, dental tissues, synovial fluid, dermis, muscle etc [22].

Thanks to their availability in various adult tissues, MSCs represent a promising tool for cell-based therapies because of their self-renewal capacity and multipotent differentiation capacity. However, for their application in experimental or clinical settings, a great number of MSCs is necessary, thus
presenting a challenge in laboratory conditions which are often related to disrupted functionality of MSCs. Previously, it has been shown that bone marrow MSCs loose replication capacity and differentiation potential during two months of *in vitro* cultivation [23]. There are opposite data about effects of extensive *ex vivo* expansion of MSCs in laboratories on their biology, where although a risk of malignant transformations was suggested [2], there is evidence that long term cultivation of MSCs could not lead to transformation, because MSCs are not able to escape senescence [17, 24, 25]. Importantly, recent data showed a key role of telomere status in HSCs and bone marrow MSCs for the development of dyskeratosis congenita, a condition where shortened telomeres not only in HSCs, but also in bone marrow MSCs lead to disease development. This study demonstrated that shortened telomeres are involved in altered colony forming ability, differentiation capacity and hematopoiesis supporting role of bone marrow MSCs [26].

**Research Highlights**

It is known that telomeres shorten during cultivation of MSCs, leading to cellular senescence and decreased proliferation rate, accompanied with shift of telomere to the nuclear center [27]. In our previously published study, we compared properties of cells isolated from human tissues: umbilical cord (UC-MSCs), peripheral blood (PB-MSCs), exfoliated deciduous teeth (SHEDs), periodontal ligament (PDL-MSCs) and adipose tissue (AT-MSCs) [28]. In the first step, our results demonstrated that all types of cells expressed mesenchymal CD markers: CD90, CD105, CD44 and CD73, while hematopoietic CD34 and CD45 were not expressed. Additionally, all types of isolated cells demonstrated capacity for colony forming in CFU-F assay. However, capacity for CFU-F forming varied: PB-MSCs (n>65), UC-MSCs (n>60), SHEDs (n>38), PDL-MSCs (n>10), AT-MSCs (n=10), thus indicating a potential difference in their self-renewal capacity.

Besides various CFU-F efficiencies, in our study isolated cells demonstrated significantly different proliferative capacities. UC-MSCs and PB-MSCs demonstrated the highest proliferation rate, while PDL-MSCs and AT-MSCs showed the lowest. In accordance with their proliferation and population doublings, isolated cells showed different relative telomere lengths (RTL) when they were compared in same passage. PB-MSCs showed the highest RTL, which was in accordance with their highest proliferation capacity. On the other hand, AT-MSCs showed the lowest RTL value, which was in accordance with their lowest proliferation rate. SHEDs and PDL-MSCs showed higher RTL values that AT-MSCs, but lower than in PB-MSC and UC-MSCs. However, although UC-MSCs showed high proliferation capacity, they had lower RTL value than PB-MSCs. Also, a similar trend was observed in RTL values between isolated cell populations, to that in CFU-F efficiency. Therefore, we can suppose that telomere status of isolated cells was the cause of their CFU-F efficiency and replicative potential, as previously suggested for bone marrow MSCs [29] and AT-MSCs [30].

Moreover, we investigated hTERT mRNA expression in all isolated cell populations. Obtained results showed that UC-MSCs and PB-MSCs expressed the highest level of hTERT mRNA in comparison to other cells. Interestingly, PDL-MSCs and AT-MSCs showed higher hTERT mRNA expression than SHEDs. Expression of hTERT mRNA was proposed as a predictive marker of telomerase activity [6]. Although we could not conclude that PDL-MSCs and AT-MSCs had higher telomerase activity, we can suppose that this level of hTERT mRNA expression was not sufficient to maintain telomere length in these cells, which finally contributed to their low replicative capacity.

It can also be speculated that the difference observed in telomere length and hTERT expression can be explained by different maturity of isolated cells. In addition, we want to point out that their differences could be related to tissue-origin, donor age and behavior of cells *in vitro*. Moreover, heterogeneity of isolated MSCs populations derived from adult tissues make comparison of their properties even more difficult [31].

As the regenerative potential of MSCs rests on their ability to generate mature cell types through processes of differentiation, these adult stem cell populations might also exhibit similar heterogeneity within their primitive compartments.

To confirm their mesenchymal stem/stromal-like nature, we investigated differentiation ability of isolated cells. Our results confirmed tri-lineage differentiation capacity of all isolated cells. Interestingly, we also observed spontaneous differentiation of isolated cells. PB-MSCs and UC-MSCs showed spontaneous adipogenic differentiation while SHEDs and PDL-MSCs demonstrated spontaneous osteogenic differentiation. AT-MSCs showed spontaneous differentiation of both. However, besides the highest RTL value and hTERT mRNA expression, PB-MSCs and UC-MSCs showed highest expression of PPARγ mRNA, major transcription factor of adipogenesis. Contrary, SHEDs, PDL-MSCs and AT-MSCs, possessed lower RTL values and hTERT mRNA expression, but higher expression of Cbfa1, major transcriptional factor of osteogenesis. Negative correlation between expression of Cbfa1 and TERT was also described in human bone marrow [32] and placental MSCs [33].
Although spontaneous differentiation of MSCs in cell culture conditions is an undesirable feature, some studies have shown that MSCs can undergo spontaneous differentiation [34] or transformation [17] during culturing. However, whether observed spontaneous differentiation is a consequence of heterogeneity of isolated MSCs or an artifact of in vitro cultivation, needs to be further elucidated.

Moreover, our results showed that SHEDs, PDL-MSCs and AT-MSCs, possessed lower mRNA expression for TERT, but higher expression of pluripotency markers mRNA (Nanog, Oct-4A and B, SOX-2) than PB-MSCs and UC-MSCs. Contrary, in previous study, hTERT has been proposed to have a role in the upregulation of pluripotency marker expression in MSCs [35]. Although expression of pluripotency markers in adult stem cells is demonstrated in many studies [33, 36], their role is still not well defined. In some studies, pluripotency marker expression was shown to be related to MSCs stemness and undifferentiated status maintenance [37], while other studies showed their overexpression was related to enhanced differentiation of MSCs [36, 38]. This complexity is probably a consequence of questionable expression of pluripotency markers in MSCs, because these markers, such as Oct4 possess several pseudogenes which share high sequence homology to Oct-4A [39] and have non-pluripotency activities. This discrepancy within expression of TERT, pluripotency markers and differentiation of MSCs opens many questions regarding the role of telomeres and telomerase in the regulation of stemness and functionality of MSCs.

Conclusions

Our study drew attention to possibility that MSCs, possess and reserve tissue origin-related individualities, which affect their native and in vitro stem-cell-like features. Although cells isolated from various tissues fulfilled the criteria for MSCs characterization and displayed similar phenotypes, they exhibited high degree of variability in their RTL and hTERT mRNA expression. This variability was in line with their replication and clonogenic capacity. Moreover, our study showed that MSCs with higher RTL and hTERT mRNA expression had higher spontaneous adipogenesis level, contrary to those which had lower telomere lengths and hTERT mRNA expression and demonstrated spontaneous osteogenesis ability. Our results led us to conclude that telomere status can be predictable factor in the evaluation of replication and self-renewal capacity of MSCs. It is possible to speculate that telomere and telomerase status in MSCs could control stemness of these cells, by regulating expression of pluripotency markers and differentiation processes. However, more detailed studies are necessary to fully reveal molecular mechanisms involved in telomere- and telomerase-mediated effects on MSCs behavior and functions.

Conflicting interests

The authors have declared that no conflict of interests exist.

Acknowledgments

This study was supported by grant #175062 from the Ministry of Education, Science and Technological Development of Republic of Serbia.

Author’s contribution


References


