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Human mesenchymal stem cells with enhanced telomerase activity acquire resistance against oxidative stress-induced genomic damage

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Abstract

Background. Human mesenchymal stem cells (MSC) are important tools for several cell-based therapies. However, their use in such therapies requires *in vitro* expansion during which MSCs quickly reach replicative senescence. Replicative senescence has been linked to macromolecular damage, and especially oxidative stress-induced DNA damage. Recent studies on the other hand, have implicated telomerase in the cellular response to oxidative damage, suggesting that telomerase has a telomere-length independent function that promotes survival. *Methods.* Here, we studied the DNA damage accumulation and repair during *in vitro* expansion as well as after acute external oxidative exposure of control MSCs and MSCs that overexpress the catalytic subunit of telomerase (hTERT MSCs). *Results.* We showed that hTERT MSCs at high passages have a significant lower percentage of DNA lesions as compared to control cells of the same passages. Additionally, less damage was accumulated due to external oxidative insult in the nuclei of hTERT overexpressing cells as compared to the control cells. Moreover, we demonstrated that oxidative stress leads to diverse nucleus malformations, such as multillobular nuclei or donut-shaped nuclei, in the control cells whereas hTERT MSCs showed significant resistance to the formation of such defects. Finally, hTERT MSCs were found to possess higher activities of the basic antioxidant enzymes, superoxide dismutase and catalase, than control MSCs. *Discussion.* On the basis of these results, we propose that hTERT enhancement confers resistance to genomic damage due to the amelioration of the cell's basic antioxidant machinery.

Key Words: *mesenchymal stem cells, telomerase, oxidative stress, genomic damage*

Abbreviations: MSCs, human Mesenchymal Stem Sells; ASCs, Adipose Stem Cells; WJ, Wharton's Jelly; hTERT, catalytic subunit of human telomerase; ROS, Reactive Oxygen Species; SAC, Spindle Assembly Checkpoint.

Introduction

Several studies have demonstrated the therapeutic potential of human mesenchymal stem cells (MSCs). Their self-renewal ability and multilineage differentiation (stemness) have made them a rather attractive candidate for cell based therapies. In fact, clinical trials are currently taking place using MSCs as the main therapeutic agent for different human diseases [1]. Even though MSCs can be easily isolated from bone marrow, adipose tissue, the umbilical cord and other tissues, their expansion *in vitro* is not free of concerns [2,3]. It is actually well documented that MSCs reachreplicative senescence rather fast in culture conditions [4,5] which impairs their regenerative potential [6,7].

Replicative senescence is a common feature to all somatic cells and most stem cells; after a period of rigorous proliferation the rate of cell division drops until cells reach a state where they are still alive but unable to divide further. The phenomenon is accompanied by a number of characteristic changes, such as increase in size and flattening, nuclear changes as well as several changes in gene and protein expression patterns [8,9]. The telomere shortening hypothesis is currently the best explanation for the limited proliferation potential of the cells. Telomeres are hexanucleotide repeats which extend

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over several thousand base pairs (bp) in length, forming functional "caps" at the end of the chromosomes. These repeats due to the "end-replication-problem" are subjected to erosion with each round of DNA replication. Telomeric DNA is synthesized by a specialized reverse transcriptase called telomerase [10]. When telomerase is not present or is expressed at very low levels, progressive shortening of the telomeres is taking place in every cell division until the telomeres reach a crucial length. At this point cellular senescence is triggered by activating the DNA damage checkpoint that prevents cells from further cycling [11].

For MSCs the proliferation limit determined by the length of their telomeres bears another obstacle: the age of the donor of the cells, since it has been shown to be correlated with the initial telomeres length, and therefore the time that MSCs can be maintained in culture [12]. The initial telomere length of the donor's MSCs could therefore be a limiting factor of the MSCs therapeutic potential since it will affect the survival and integration ability of the transplanted cells to the adult tissue [13–15]. In fact there is increasing evidence that the age of the donor affects several properties of MSCs [16].

On the other hand, it has been demonstrated that the erosion of the telomeres is not only due to the way DNA is replicated, but it can also be accelerated or even induced by environmental factors, such as the oxidative stress (OS) [17,18]. Different human cells subjected to long-term exposure to mild OS have shown to undergo accelerated telomere erosion that triggers premature senescence [17]. This phenomenon has been collectively termed 'stress induced premature senescence' (SIPS) [19]. It was also reported that hydrogen peroxide (H_2O_2) can cause predominant DNA damage at the 5′ site of 5′-GGG-3′ in the telomere sequence. Furthermore, H_2O_2 induced the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG), an oxidized derivative of deoxyguanosine and major product of DNA oxidation [20], in telomere sequences more efficiently than that in non-telomere sequences [21]. Different groups, however, have reported that SIPS could also be independent of telomeric damage [22]. Despite the controversies on the matter, it is widely acknowledged that the progressive oxidative damage to the macromolecules, and especially to DNA, can induce cellular senescence [23]. Even though many different groups have studied the involvement of OS in MSCs senescence in culture [24–26], the exact underlying molecular mechanism remains unclear.

Interestingly, it has been recently reported that telomerase has also non-canonical or extratelomeric functions, that are involved in processes such resistance to stress and especially to oxidative stress. In fact, Borras *et al.* [27] showed that glutathione, a physiological antioxidant regulates telomerase activity, highlighting therefore the interplay between telomerase and the cellular oxidative status. Moreover, emerging data demonstrate that telomerase could be directly involved in the reduction of reactive oxygen species (ROS) generation and therefore decreased oxidative DNA damage [28–31].

In order to investigate the antioxidant properties of telomerase, and their implication in the DNA damage driven decline in the cellular proliferation potential, in the present study we analyzed the oxidative stress-induced DNA damage response of MSCs genetically modified with the catalytic subunit of human telomerase (hTERT). In the analysis we included adipose derived MSCs (ASCs) from adult individuals as well as umbilical cord's Wharton Jelly derived cells (WJ-MSCs), in an attempt to determine possible differences that could be attributed to the donor's initial telomeres length. We believe that this approach provides insights regarding the recently proposed antioxidant role for telomerase, which could contribute in developing strategies that will overcome the senescence-associated impairment of the regenerative potential of MSCs.

Materials and methods

Construction of the hTERT transposon

An entry clone encoding the human telomerase reverse transcriptase catalytic subunit (hTERT), (GeneID: 7015, clone ID: IOH36343) was shuttled into a pT2-CAGGS-EYFP-GW plasmid [32] using LR clonase (Life Technologies), according to manufacturer's instructions. The reaction mixture was used for transformation of Mach1 E. coli cells and transformants were selected on agar plates supplemented with 100 μg/ml Ampicillin. Plasmid isolation was performed using NucleoSpin plasmid isolation kit (Macherey-Nagel). The identity of pT2-CAGGS-EYFP-hTERT plasmid was verified by BsrGI restriction digestion.

Generation of hTERT mesenchymal stem cells

MSCs were enzymatically isolated from adipose tissue (ASCs) of healthy donors (four different individuals, n = 4, age range of 49–60 years) by lipoaspiration. Informed consent was given for the collection of samples from all donors and the collection was performed in accordance with established guidelines. For the isolation of ASCs from the adipose tissue, tissues were treated with collagenase (2.7 mg/ml) and hyaluronidase (0.7 mg/ml) solution for 1h at 37°C followed by incubation with trypsin (2.5%). Cell suspension was diluted with equal volume of PBS, passed through a sterile 0.2 μm filter and centrifuged at 500g for 30 min at room temperature (RT). We also isolated and used MSCs from the Wharton Jelly of umbilical cords (WJ