Base excision repair but not DNA double-strand break repair is impaired in aged human adipose-derived stem cells

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Abstract
The decline in DNA repair capacity contributes to the age-associated decrease in genome integrity in somatic cells of different species. However, due to the lack of clinical samples and appropriate tools for studying DNA repair, whether and how age-associated changes in DNA repair result in a loss of genome integrity of human adult stem cells remains incompletely characterized. Here, we isolated 20 eyelid adipose-derived stem cell (ADSC) lines from healthy individuals (young: 10 donors with ages ranging 17–25 years; old: 10 donors with ages ranging 50–59 years). Using these cell lines, we systematically compared the efficiency of base excision repair (BER) and two DNA double-strand break (DSB) repair pathways—nonhomologous end joining (NHEJ) and homologous recombination (HR)—between the young and old groups. Surprisingly, we found that the efficiency of BER but not NHEJ or HR is impaired in aged human ADSCs, which is in contrast to previous findings that DSB repair declines with age in human fibroblasts. We also demonstrated that BER efficiency is negatively associated with tail moment, which reflects a loss of genome integrity in human ADSCs. Mechanistic studies indicated that at the protein level XRCC1, but not other BER factors, exhibited age-associated decline. Overexpression of XRCC1 reversed the decline of BER efficiency and genome integrity, indicating that XRCC1 is a potential therapeutic target for stabilizing genomes in aged ADSCs.

KEYWORDS
adipose-derived stem cells, base excision repair, genome integrity, human aging, XRCC1

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1 | INTRODUCTION, RESULTS, AND DISCUSSION

The stem cell theory of aging assumes that age-associated changes in adult stem cell functionality lead to a failure to preserve tissue homeostasis, eventually resulting in the onset of aging and age-associated diseases (Goodell & Rando, 2015). As such, it is possible that stem cell therapy can benefit patients who suffer from age-related diseases such as musculoskeletal disorders (Jo et al., 2014), neurological disorders (Lee et al., 2010), and cardiovascular diseases (De Luca et al., 2019; Patel et al., 2016). Indeed, a number of clinical trials are being conducted to treat age-associated diseases using adult mesenchymal stem cells worldwide (Jo et al., 2014; Lee et al., 2010; Patel et al., 2016). The application of autologous stem cells in the clinic has received considerable attention as this method not only avoids ethical concerns associated with embryonic stem cells but also minimizes the risks of immune rejections. However, the extent to which the genome integrity of adult stem cells isolated from older patients influences the efficacy and safety outcomes of autologous stem cell therapy remains unclear. Accumulating evidence indicates that aged stem cells exhibit genomic instability (Burkhalter, Rudolph, & Sperka, 2015), possibly due to a reduced capacity for repairing DNA damage induced by either endogenous or exogenous stimuli (Park, Jeon, Pyo, Kim, & Yoo, 2018; Rossi et al., 2007). However, most of this research has been conducted using DNA repair deficient mouse models or clinical samples donated by human progeria patients (Zhang et al., 2011). Whether and how physiological aging impacts genome integrity and DNA repair in adult stem cells has not been well characterized.

The major obstacles to fully understanding the link between changes in DNA repair and adult stem cell aging include the difficulty to (a) acquire adult human stem cells from a sufficient number of donors at different ages, to (b) choose appropriate types of adult stem cells for in vitro culture, and to (c) quantify the efficiency of different types of DNA repair using reliable but easy-to-score tools. Here, we successfully obtained 20 eyelid adipose-derived stem cell (ADSC) lines from two groups of donors (a young group with ages ranging 17–25 years and an old group with ages ranging 50–59 years). Using these ADSCs, we systematically compared the efficiency of base excision repair (BER), nonhomologous end joining (NHEJ), and homologous recombination (HR) repair using our well-established plasmid reactivation assay based on a GFP gene (Li et al., 2016; Mao, Jiang, Liu, Seluanov, & Gorbonova, 2009; Xu et al., 2015). Surprisingly, we found that BER efficiency but not the other two pathways for repairing DNA DSBs declines with age in human ADSCs. We also identified that XRCC1 but not other BER factors changes with age. Overexpression of XRCC1 in old ADSCs restored the decline in BER efficiency and genome integrity.

Unlike other types of adult stem cells, ADSCs can be easily obtained with minimal harm to donors and expanded in vitro with well-established procedures (Chen et al., 2016). By analyzing the differentiation potential and surface markers, we validated the procedure for isolating ADSCs. We found that the isolated cells held the potential to differentiate into both adipocytes and osteocytes (Figure S1 A). In addition, consistent with previous reports (Locke, Windsor, & Dunbar, 2009), flow cytometry analysis indicated that the isolated ADSCs were CD13, CD29, CD44,
(a) Tail moment

(b) Tail moment

(c) pEGFP-N1 → Damaged pEGFP-N1 → Purification and transfection → BER → Repaired pEGFP-N1

- Methylene Blue
- 8-oxo-G

(d) Relative BER efficiency (GFP+/DsRed+)

(e) Relative BER efficiency (GFP+/DsRed+)

(f) Relative BER efficiency (GFP+/DsRed+) vs. Tail moment

(g) Survival fraction (%) vs. MMS (mM)

(h) MMS-LD50 (mM) vs. Young vs. Old

(i) Relative BER efficiency (GFP+/DsRed+) vs. MMS-LD50 (mM)
CD90, and CD105 positive, but CD14, CD31, and CD45 negative (Figure S1 B). These results demonstrated that we successfully isolated ADSCs.

We then isolated 20 eyelid ADSC lines from donors undergoing plastic surgery (Figure S1 C). Upon isolation of the cells, we observed ~50–100 colonies of ADSCs from each cell line. When these primary ADSCs reached confluence in a 10-cm plate, the cell numbers were ~1–2 × 10^5. Therefore, the starting population doublings (PDs) for these cell lines were between ~13 and 15. Since DNA repair capacity may change with the increase in cumulative PDs (Mao et al., 2012; Seluanov, Mittelman, Pereira-Smith, Wilson, & Gorbunova, 2004), we performed all the following experiments at the PD 23–25 in all cell lines (Figure S1 C). In addition, we compared the purity of the isolated ADSCs. Expression of three ADSC markers, CD13, CD44, and CD105 was tested in all isolated ADSCs at PD 23–25. We found that over 90% of the cells stained positive for the ADSC markers in all the 20 cell lines; we did not observe any significant difference between the two groups of cells (Figure S1 d–e).

To compare the difference in genome integrity between the two groups of ADSCs, we first performed alkaline comet assays. The average tail moment of the 10 old ADSCs was 3.3-fold higher than that of the 10 young ADSCs (Figure 1a), and the statistical analysis indicates that the tail moment of the old group of ADSCs was significantly higher than that of the young group (Figure 1b), suggesting that age has a negative impact on genome integrity.

We therefore set out to examine whether an impairment in DNA repair capacity contributes to the loss of genome integrity. Since mounting evidence indicates that the efficiency of two DNA DSB repair pathways—NHEJ and HR declines with age in somatic cells in both mice and human beings (Li et al., 2016; Vaidya et al., 2014) and that deficiency in NHEJ or HR pathway leads to a phenotype of premature aging (Espejel et al., 2004; White & Vijg, 2016), we therefore first examined if NHEJ or HR changed with age in human ADSCs using our well-characterized, GFP-based reporter cassettes (Li et al., 2016; Mao et al., 2009). We linearized NHEJ or HR reporter cassettes with the restriction enzyme, I-SceI in vitro and then transfected the digested NHEJ (0.6 µg) or HR (1.5 µg) reporter constructs together with 0.03 µg pCMV-DsRed2, for normalizing transfection efficiency, into each exponentially proliferating ADSC line at a concentration of 8 × 10^5 cells/ transfection. FACS analysis was performed on day 3 post-transfection. Surprisingly, we did not observe any difference in either NHEJ efficiency (Figure S2 a–c) or HR efficiency (Figure S2 d–f) between the two groups of ADSCs. Furthermore, we analyzed the foci formation of γH2AX and 53BP1, two markers for DSBs, at different time points postionizing radiation (IR) in the young and old ADSC lines. We found that, consistent with the results of NHEJ and HR efficiency, the formation and clearance of γH2AX and 53BP1 foci did not differ between the two groups of ADSC lines (Figure S3 a–f), strongly indicating that adult stem cells retain their ability to repair DNA DSBs during aging.

We next tested if age impacted BER in ADSCs using plasmid reactivation assays (Xu et al., 2015). We treated the pEGFP-N1 plasmid with methylene blue and visible light to induce 7,8-dihydroxy-8-oxoguanine and other types of single base modifications (Figure 1c) (Xu et al., 2015), followed by transfection of 0.15 µg of damaged pEGFP-N1 together with 0.03 µg pCMV-DsRed2. On day 3 post-transfection, we scored the number of GFP+ and DsRed + cells and employed the ratio of GFP+/DsRed+ as a measure of BER efficiency (Xu et al., 2015). By comparing the BER efficiency between the two groups of ADSCs, we found that BER efficiency in the old group of ADSCs was significantly lower than that in the young group (Figure 1 d–e). To rule out the possibility that the state of quiescence or proliferation might affect the results of the plasmid reactivation assay for measuring BER efficiency, we analyzed BER efficiency in the quiescent state in two young (Y-7 and Y-10) and two old (O-1 and O-8) ADSC lines. We found that in the quiescent state, the two young ADSCs had significantly higher BER efficiency than the two old ADSCs (Figure S4 a–b), which is in agreement with the results acquired from exponentially proliferating cells.

We then examined the correlation between BER efficiency and tail moment. We observed a significant negative correlation between BER efficiency and tail moment (Figure 1f), indicating that the age-associated decline in BER contributes to the loss of genome integrity observed in old ADSCs. Moreover, we further examined the functional consequences of this age-associated decline in BER efficiency in ADSCs. As defects in BER sensitize cells to reactive oxygen species or alkylating agents (Bauer et al., 2011; Kondo, Takahashi, Ono, & Ohnishi, 2010), we treated cells from each ADSC line with increasing concentrations of the alkylating agent methylmethane sulfonate (MMS) and performed clonogenic assays. Afterwards, we calculated LD50 and found that indeed, the aged group of cells was more sensitive to MMS than the young group (Figure 1 g–h and Figure S5). We also observed a positive correlation between LD50 and BER efficiency (Figure 1i).

To understand what led to the impairment of BER in old ADSCs, we extracted proteins from the 20 ADSC lines and analyzed the protein level of multiple BER factors including XRCC1, DNA Lig3, PARP1, APE1, OGG1,POLβ, and NTHL1. We found that only XRCC1 protein level was significantly downregulated in the old group in comparison to the young group of ADSCs (Figure 2a,b), while the other six BER factors did not change at the protein level with age.

**FIGURE 2** XRCC1 is a critical factor regulating age-associated decline in BER efficiency and genome integrity. (a) Western blot analysis of the expression of all BER factors across the 20 cell lines. All cells were harvested and lysed for protein extraction on day 2 post-splitting, when cells were exponentially proliferating. (b) Mann–Whitney U test analysis indicates that the XRCC1 protein level in the young group of ADSCs is significantly higher than that in the old group. (c) Analysis of the correlation between XRCC1 protein level and BER efficiency. (d and e) Representative pictures (d) and statistical analyses (E) of the rescue of BER efficiency by XRCC1 overexpression in the O-1, O-3, O-6, and O-8 ADSC lines. Results are presented as mean ± SD, and Student’s t-test is used for statistical analysis. (f and g) Representative pictures (f) and statistical analysis (g) of the rescue of genome integrity by XRCC1 overexpression in the O-1, O-3, O-6, and O-8 ADSC lines. Results are presented as mean ± SEM and Student’s t-test is used for statistical analysis. (h) Western blot analysis of XRCC1 level in O-1, O-3, O-6, and O-8 ADSC lines with XRCC1 overexpressed.
(a) Western blot analysis of various proteins including XRCC1, LIG3, PARP1, APE1, OGG1, POLβ, and TUBULIN across different age groups (Y-1 to Y-10 for young and O-1 to O-10 for old).

(b) Graph showing relative XRCC1 protein levels, with Y-axis labeled as p < 0.001.

(c) Scatter plot illustrating the relationship between relative XRCC1 protein level and relative BER efficiency (GFP+/DsRed+), with r² = 0.2049 and p < 0.05.

(d) Flow cytometry graphs for pcDNA and XRCC1 showing DsRed/GFP percentages.

(e) Bar graph depicting relative BER efficiency (GFP+/DsRed+) with p-values indicated for each comparison.

(f) Fluorescence images of pcDNA and XRCC1 showing green fluorescence.

(g) Tail moment graph with p-values for each condition.

(h) Western blot images of XRCC1 and TUBULIN with age groups O-1 to O-8.
Here, for the first time, with a collection of ADSC lines isolated from different donors, we demonstrated that age has a negative effect on the BER pathway, but not either of the major DSB repair pathways. Our data also indicate that targeting XRCC1 is a promising method to stabilize genomes and improve the functionality of ADSCs. However, whether improving genome integrity by activating the BER pathway directly improves ADSC functionality and what causes the age-related decline in XRCC1 protein level are questions that remain to be determined.

ACKNOWLEDGMENT

This research was funded by the National Key R&D Program of China (2018YFC2000100, 2017YFA0103300), the National Science Foundation of China (81622019, 31871438, 31570813, 81702770, 81702638 and 81601212), Program of Shanghai Academic Research Leader (19XD1403000), the Fundamental Research Funds for the Central Universities, the Open Project Program of State Key Laboratory of Natural Medicines (SKLNMKF201905), the Shanghai municipal medical and health discipline construction projects (No. 2017ZZ20015) and the “Shu Guang” project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (19SG18).

CONFLICT OF INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

Haiping Zhang and Bailian Cai were involved in ADSC isolation, cell culture, cell differentiation, DNA repair analysis, comet assay, manuscript writing, data analysis, and interpretation. Anke Geng and Sheng Li were involved in data analysis and interpretation, and collection and assembly of data. Huanyin Tang was involved in statistical analysis and graphing. Wenjun Zhang was involved in collection of clinical samples. Ying Jiang assisted with the provision of study material and collection of data. Rong Tan, Xiaoping Wan, and Zhiyong Mao were involved in the conception and design, data interpretation, manuscript writing, and final approval of manuscript.

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REFERENCES

Bauer, M., Goldstein, M., Christmann, M., Becker, H., Heylmann, D., & Kaina, B. (2011). Human monocytes are severely impaired in base and DNA double-strand break repair that renders them vulnerable to oxidative stress. Proc Natl Acad Sci U S A, 108(52), 21105–21110. https://doi.org/10.1073/pnas.1111919109
Burkhalter, M. D., Rudolph, K. L., & Sperka, T. (2015). Genome instability of ageing stem cells—Induction and defence mechanisms. Ageing Research Reviews, 23(Pt A), 29–36. https://doi.org/10.1016/j.arr.2015.01.004

Caldecott, K. W., McKeown, C. K., Tucker, J. D., Ljungquist, S., & Thompson, L. H. (1994). An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. Molecular and Cellular Biology, 14(1), 68–76. https://doi.org/10.1128/mcb.14.1.68

Chen, Y. J., Liu, H. Y., Chang, Y. T., Cheng, Y. H., Mersmann, H. J., Kuo, W. H., & Ding, S. T. (2016). Isolation and Differentiation of Adipose-Derived Stem Cells from Porcine Subcutaneous Adipose Tissues. Journal of Visualized Experiments, 109, e53886. https://doi.org/10.3791/53886

De Luca, M., Aiuti, A., Cossu, G., Parmar, M., Pellegrini, G., & Robey, P. G. (2019). Advances in stem cell research and therapeutic development. Nature Cell Biology, 21(7), 801–811. https://doi.org/10.1038/s41556-019-0344-z

Espejel, S., Martin, M., Klatt, P., Martin-Caballero, J., Flores, J. M., & Blasco, M. A. (2004). Shorter telomeres, accelerated ageing and increased lymphoma in DNA-PKcs-deficient mice. EMBO Reports, 5(5), 503–509. https://doi.org/10.1038/sj.embor.7400117

Fang, Q., Inanc, B., Schamus, S., Wang, X., Wei, L., Brown, A. R., ... Sobol, R. W. (2014). HSP90 regulates DNA repair via the interaction between XRCC1 and DNA polymerase β. Nature Communications, 5(1).

Goodell, M. A., & Rando, T. A. (2015). Stem cells and healthy aging. Science, 350(6265), 1199–1204. https://doi.org/10.1126/science.aab3388

Jo, C. H., Lee, Y. G., Shin, W. H., Kim, H., Chai, J. W., Jeong, E. C., ... Yoon, K. S. (2014). Intrarticular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: A proof-of-concept clinical trial. Stem Cells, 32(5), 1254–1266. https://doi.org/10.1002/stem.1634

Kang, H. C., Lee, Y.-I., Shin, J.-H., Andrabi, S. A., Chi, Z., Gagne, J.-P., ... Dawson, T. M. (2011). Iduna is a poly(ADP-ribose) (PAR)-dependent E3 ubiquitin ligase that regulates DNA damage. Proceedings of the National Academy of Sciences, 108(34), 14103–14108. https://doi.org/10.1073/pnas.1108799108

Kondo, N., Takahashi, A., Ono, K., & Ohnishi, T. (2010). DNA damage induced by alkylating agents and repair pathways. Journal of Nucleic Acids, 2010, 543531. https://doi.org/10.4062/2010/543531

Lee, J. K., Jin, H. K., Endo, S., Schuchman, E. H., Carter, J. E., & Bae, J. S. (2010). Intracerebral transplantation of bone marrow-derived mesenchymal stem cells reduces amyloid-beta deposition and rescues memory deficits in Alzheimer’s disease mice by modulation of immune responses. Stem Cells, 28(2), 329–343. https://doi.org/10.1002/stem.277

Li, Z., Zhang, W., Chen, Y., Guo, W., Zhang, J., Tang, H., ... Mao, Z. (2016). Impaired DNA double-strand break repair contributes to the age-associated rise of genomic instability in humans. Cell Death and Differentiation, 23(11), 1765–1777. https://doi.org/10.1038/cdd.2016.65

Liu, M., Lei, H., Dong, P., Fu, X., Yang, Z., Yang, Y., ... Xiao, R. (2017). Adipose-Derived Mesenchymal Stem Cells from the Elderly Exhibit Decreased Migration and Differentiation Abilities with Senescent Properties. Cell Transplantation, 26(9), 1505–1519. https://doi.org/10.1177/0963689717721221

Locke, M., Windsor, J., & Dunbar, P. R. (2009). Human adipose-derived stem cells: Isolation, characterization and applications in surgery. ANZ Journal of Surgery, 79(4), 235–244. https://doi.org/10.1111/j.1445-2197.2009.04852.x

Mao, Z., Jiang, Y., Liu, X., Seluanov, A., & Gorbunova, V. (2009). DNA repair by homologous recombination, but not by nonhomologous end joining, is elevated in breast cancer cells. Neoplasia, 11(7), 683–691. https://doi.org/10.1593/neo.09312

Mao, Z., Tian, X., Van Meter, M., Ke, Z., Gorbunova, V., & Seluanov, A. (2012). Sirtuin 6 (SIRT6) rescues the decline of homologous recombination repair during replicative senescence. Proceedings of the National Academy of Sciences, 109(29), 11800–11805. https://doi.org/10.1073/pnas.1200583109

Park, J. S., Jeon, H. J., Pyo, J. H., Kim, Y. S., & Yoo, M. A. (2018). Deficiency in DNA damage response of enterocytes accelerates intestinal stem cell aging in Drosophila. Aging (Albany NY), 10(3), 322–338. https://doi.org/10.18632/aging.103390

Parsons, J. L., Tait, P. S., Finch, D., Dianova, I. I., Allinson, S. L., & Dianov, G. L. (2008). CHIP-mediated degradation and DNA damage-dependent stabilization regulate base excision repair proteins. Molecular Cell, 29(4), 477–487. https://doi.org/10.1016/j.molcel.2007.12.027

Parsons, J. L., Tait, P. S., Finch, D., Dianova, I. I., Edelman, M. J., Khoronkova, S. V., ... Dianov, G. L. (2009). Ubiquitin ligase ARF-BP1/Mule modulates base excision repair. EMBO Journal, 28(20), 3207–3215. https://doi.org/10.1038/embj.2009.243

Patel, A. N., Henry, T. D., Quyyumi, A. A., Schaer, G. L., Anderson, R. D., Toma, C., ... DeMaria, A. (2016). Ixmyelocel-T for patients with ischaemic heart failure: A prospective randomised double-blind trial. Lancet, 387(10036), 2412–2421. https://doi.org/10.1016/s0140-6736(16)30137-4

Rossi, D. J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., & Weissman, I. L. (2007). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature, 447(7145), 725–729. https://doi.org/10.1038/nature05862

Seluanov, A., Mittelman, D., Pereira-Smith, O. M., Wilson, J. H., & Gorbunova, V. (2004). DNA end joining becomes less efficient and more error-prone during cellular senescence. Proceedings of the National Academy of Sciences, 101(20), 7624–7629. https://doi.org/10.1073/pnas.0400726101

Vaidya, A., Mao, Z., Tian, X., Spencer, B., Seluanov, A., & Gorbunova, V. (2014). Knock-in reporter mice demonstrate that DNA repair by non-homologous end joining declines with age. PLoS Genetics, 10(7), e1004511. https://doi.org/10.1371/journal.pgen.1004511

Valverde, M., Lozano-Salgado, J., Fortini, P., Rodriguez-Sastre, M. A., Rojas, E., & Dogliotti, E. (2018). Hydrogen Peroxide-Induced DNA Damage and Repair through the Differentiation of Human Adipose-Derived Mesenchymal Stem Cells. Stem Cells International, 2018, 1615497. https://doi.org/10.1155/2018/1615497

White, R. R., & Vijg, J. (2016). Do DNA Double-Strand Breaks Drive Aging? Molecular Cell, 63(5), 729–738. https://doi.org/10.1016/j.molcel.2016.08.004

Xu, Z., Zhang, L., Zhang, W., Meng, D. U., Zhang, H., Jiang, Y., ... Mao, Z. (2015). SIRT6 rescues the age related decline in base excision repair in a PARP1-dependent manner. Cell Cycle, 14(2), 269–276. https://doi.org/10.4161/15384101.2014.980641

Zhang, J., Lian, Q., Zhu, G., Zhou, F., Sui, L., Tan, C., ... Colman, A. (2011). A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects. Cell Stem Cell, 8(1), 31–45. https://doi.org/10.1016/j.stem.2010.12.002

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