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Research Paper

NAC selectively inhibit cancer telomerase activity: A higher redox homeostasis threshold exists in cancer cells

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ABSTRACT

Telomerase activity controls telomere length, and this plays an important role in stem cells, aging and tumors. Antioxidant was shown to protect telomerase activity in normal cells but inhibit that in cancer cells, but the underlying mechanism is elusive. Here we found that 7721 hepatoma cells held a higher redox homeostasis threshold than L02 normal liver cells which caused 7721 cells to have a higher demand for ROS; MnSOD over-expression in 7721 decreased endogenous reactive oxygen species (ROS) and inhibited telomerase activity; Akt phosphorylation inhibitor and NAC both inhibited 7721 telomerase activity. The over-elimination of ROS by NAC resulted in the inhibition of Akt pathway. Our results suggest that ROS is involved in the regulation of cancer telomerase activity through Akt pathway. The different intracellular redox homeostasis and antioxidant system in normal cells and tumor cells may be the cause of the opposite effect on telomerase activity in response to NAC treatment. Our results provide a theoretical base of using antioxidants selectively inhibit cancer telomerase activity. Findings of the present study may provide insights into novel approaches for cancer treatment.

1. Introduction

Telomeres are nucleoprotein structures, located at the ends of chromosomes and are subject to shortening at each cycle of cell division, which forces human primary cells to stop dividing when a critical minimum telomere length is reached [1,2]. They are synthesized by telomerase consisting of a reverse transcriptase catalytic subunit (TERT) and an RNA template subunit (TERC) [3,4]. Telomerase activity is closely related to aging and tumorigesis. It is generally inhibited in normal cells but highly activated in tumor cells. The differential expression of the telomerase enzyme in normal and cancer cells have led to the evolution of tumor specific anti-telomerase approaches which inhibit the telomerase enzyme activity so as to destabilize and shorten the telomeres, leading to senescence in cancer cells.

In numerous studies on telomerase regulation mechanism, the relationship between oxidative stress and telomerase has successfully attracted worldwide attention. As reactive oxygen species (ROS) are particularly injurious toward the G-rich sequences of telomeres [5], ROS scavengers, also called antioxidants, have been used in protecting telomere and slowing the aging process of cells for many years [2,6,7]. However, in tumor cells, antioxidants exhibit quite the opposite effect. Many drugs which have been reported to possess antioxidant properties could inhibit tumor telomerase activity, promoting telomere shortening and provoking tumor cell apoptosis [8–12]. To the best of our knowledge, there is no research has explained the reason why antioxidants have different effects on telomerase activity of normal and tumor cells, and the present study is trying to explore the potential mechanism underlying this phenomenon.

Antioxidants such as NAC directly affect both ROS levels and the intracellular redox state (REDST). The intracellular oxidative damage/antioxidant defense is maintained at a relatively constant state relying on the combinatory effects of a variety of oxidizing substances, antioxidants and other related enzymatic systems. Redox disorders would cause redox balance to be shifted to the direction of oxidation or reduction, leading to excessive ROS production or elimination. Both excessive and insufficient amount of ROS will bring about bad effect on health, as ROS are no longer viewed just as a toxic by-product of mitochondrial respiration, but a double-edge sword and play an important role in numerous cellular processes. There is a growing body of evidence have indicated that ROS may be an essential element required for regulating a myriad of signaling pathways [13]. In consideration that ROS has both positive and negative effect, it is conceivable that the different intracellular redox state in different types of cell confer themselves different redox balance, and developed different sensitivity to oxidative stress or antioxidant interference, which could

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be the potential reason why telomerase activity of normal cells and cancer cells respond differently to anti-oxidative drugs.

In the present study we took normal liver cell line L02 and liver cancer cell line SMMC-7721 as research object, trying to uncover the reason why antioxidants inhibit cancer cells, and explore the role played by antioxidant system and Akt signaling pathway in regulating cancer cell's telomerase activity.

2. Materials and methods

2.1. Cell culture and hypoxia exposure

L02 and SMMC-7721 cell lines were obtained from Shanghai Institute of Cell Biology. Cells were grown in DMEM (Hyclone) supplemented with 2 mM L-glutamine and 10% FBS in a humidified incubator at 37 °C and 5% CO_2. Hypoxia exposures were done in a tri-gas tissue culture incubator (Binder) which could adjust the oxygen concentration to 5%, 2% and 0.5%.

2.2. MTT assay

1×10^4 cells were seeded in triplicate in a 96-well plate in a final volume of 100 μl and incubated for 4 h. Cells treated with DMSO alone were used as controls. Intervention buffer (100 μl) was then added and cultured the cells for the indicated times. At the end of the treatment, 10 μl MTT (5 mg/ml) was added to each well and incubated for an additional 4 h at 37 °C. DMSO (100 μl)/well were added after dropping the old medium with MTT. The absorbance was measured at 570 nm using a microplatereader (Biotek Synergy 4).

2.3. GSH assay

Cells were scraped into 50 μl 1 M HPO_3 and freeze/thaw for 2–3 cycles, the suspension was centrifuged with 12,000 rpm for 10 min at 4 °C and then assayed as previously described [14].

2.4. Telomerase activity assay

Extracts of cell lines were prepared as described previously [15] and the lysis buffer contained 10 mM Tris–HCl, pH 8.0; 1 mM $MgCl_2$; 1 mM EDTA; 1% (vol/vol) NP-40; 0.25 mM sodium deoxycholate; 10% (vol/vol) glycerol; 150 mM NaCl; 5 mM β-mercaptoethanol, PMSF. The extracts were quickly frozen in liquid nitrogen and stored at −80 °C. The extracts were centrifuged at 13,000 rpm for 30 min at 4 °C. The supernatant was used for protein quantification and the subsequent testing. Telomerase assay was done according to the protocol previously reported [15] with some modification. 50 μl TRAP reaction mixture containing 2 μg cell protein extracts, 5 μL 10 × TRAP buffer, 1 μL dNTPs, 0.5 μL Phusion-DNA polymerase, 1 μL TS primer (all from Thermo Scientific). After 30 min incubation at 25 °C for TS elongation by telomerase, the reaction was stopped by heating at 90 °C for 3 min. Then 1 μl CX primer was added and subjected to 35 PCR cycles at 94 °C for 10 s, 50 °C for 30 s, and 72 °C for 1 min. Another 10 min at 72 °C was needed for extention. 50 μL of PCR product was loaded and run electrophoresis in Trisborate-EDTA on 8% polyacrylamide nondenaturing gel. The gel was stained in low concentration of ethidium bromide (0.5 μg/mL) for 15 min, and then photographed under UV light.

2.5. ROS detection

For measurement of intracellular ROS, cells were harvested and incubated with 10 μM DCFH-DA (2′-, 7′-dichlorofluorescein diacetate, Sigma) for 30 min at 37 °C. Cell suspension solution was centrifuged with 3000 rpm for 5 min, and washed twice with PBS. The fluorescence intensity was analyzed by FC 500 MCL system (Beckman coulter) immediately at excitation/emission wavelength of 488 nm/525 nm.

2.6. Plasmids and transfection

For overexpression of MnSOD to downregulate ROS levels, or inhibtion of MnSOD to increase ROS level, plasmids containing sense or antisense cDNA of human MnSOD were used. pHβ A-SOD (+) or pHβ A-SOD(−) plasmids (kindly provided by Professor Kunitaka Hirose) were transfected into SMMC-7721 cells and establish human SMMC-7721 hepatoma cell lines with stable expression of MnSOD or with suppressed expression of MnSOD using a standard method as described before [16].

2.7. Immunoblotting

Total cell extracts or nuclear extracts were separated by SDS-PAGE and transferred to PVDF membranes. The following antibodies were used for immunoblot analysis: rabbit phospho-Akt (Thr308), Akt antibody and the PI3K inhibitor LY294002 were from Calbiochem and secondary antibody was purchased from Proteintech. Anti-β-actin antibody was from Cell Signaling Technology.

2.8. Statistical analysis

The results are reported as means ± standard error. Statistical significance was determined using Student's *t*-test and ANOVA, with a value of $p < 0.05$ being considered significant.

3. Results

3.1. L02 and 7721 cells need different redox state to reach their respective maximum cell viability and telomerase activity

Using cell viability as the vertical axis, and using H_2O_2 and NAC concentration as the horizontal axis respectively, a bell-shaped line (as shown in Fig. 1A) could be obtained. Such curve can also be utilized to indicate the relationship between the cellular ROS concentration and cell viability. Interestingly, the top-points of the bell curve of L02 and 7721 appeared at different positions, which indicated their different sensitivity towards the exogenous ROS. L02 cell viability reached a maximum when the concentration of NAC was 5 mM, while the 7721 cell viability reached a maximum when H_2O_2 was 5 μM.

It has been well reported that telomerase activity in normal somatic cells was basically repressed, but are highly activated in embryonic stem cells or tumor cells [15]. Given the close relationship between telomerase activity and cell viability, we suspected that telomerase activity might also represent in the form of "bell-shaped" curve when extracellular redox state changes. As expected, telomerase activity in L02 cells decreased with increasing exogenous H_2O_2 concentration, but increased as NAC concentration was raised. However, 7721 telomerase activity, on the contrary, declined (Fig. 1B). 7721 telomerase activity reached its maximum value when H_2O_2 concentration was around 5 μM, but a further increment in H_2O_2 concentration led to an inevitable decline in its activity.

3.2. Moderate hypoxia-induced oxidative stress increases 7721 cells' telomerase activity

Hypoxia is widely found in solid tumors and is the main cause

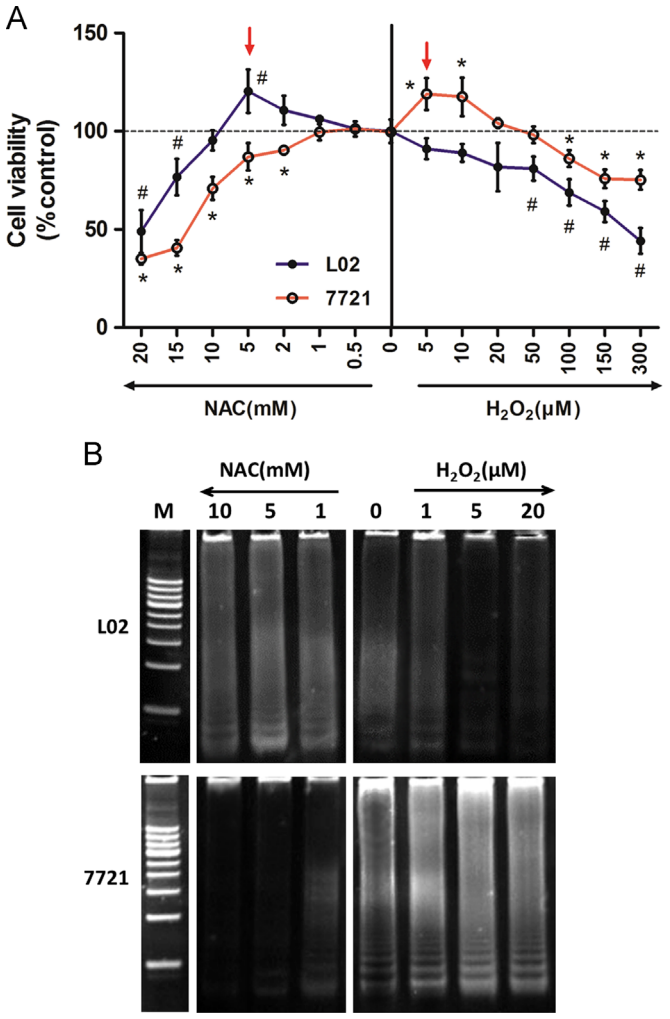

Fig. 1. Cell viability and Telomerase activity in response to NAC and H_2O_2 intervention. (A) Cell viability of L02 and 7721 cells under NAC and H_2O_2 intervention. Cells were cultured at 37 °C under 5, 10, 20, 50, 100, 150, 300 μM H_2O_2 or 0.5, 1, 2, 5, 10, 15, 20 mM NAC for 24 h before MTT was assayed. The arrows mark the top-points of the bell-shaped curve which present the highest value of cell viability. Results shown represent mean ± SD, $n=3$, $^*p<0.05$ vs. L02 control group, $\#p<0.05$ vs. 7721 control group. (B) Telomerase activity of L02 (upper row) and 7721 (bottom row) cells under NAC and H_2O_2 intervention. Cells were cultured at 37 °C under 1, 5, 20 μM H_2O_2 or 1, 5, 10 mM NAC for 24 h and then quickly collected in lysis buffer. EB-TRAP assay was conducted and the gel was photographed under UV light. The brighter the strip is, the higher the telomerase activity is in the cell sample tested.

of the high level of oxidative stress in tumor [17]. We then using hypoxia model further verified that cancer cells preferred an oxidative environment, and cells under a near-threshold redox state held the highest telomerase activity. With the improvement in the degree of hypoxia, ROS production in 7721 cells gradually increased (Fig. 2A). The telomerase activity of 7721 cells reached its highest value when oxygen content was reduced to 2%, but a further decline in oxygen content (below 2%) caused telomerase activity to decline (Fig. 2B and C).

3.3. Moderate H_2O_2 increases 7721 cells' GSH/GSSG ratio

The GSH/GSSG ratio is widely used to reflect the intracellular redox state and measure the cell's ability to fight against oxidative stress. We detected the GSH/GSSG ratio under different extracellular redox intervention. Our results showed that 7721 hepatoma cells had significantly higher GSH/GSSG ratio than L02 liver cells (Fig. 3), which indicates that the antioxidant ability of cancer cells is higher than that of normal cells under regular culture condition. Moderate H_2O_2 intervention (≤ 1 μM) increased the GSH/GSSG of both L02 and 7721, but with increment of H_2O_2 concentration, GSH/GSSG ratio of L02 declines faster compared to that of 7721. Interestingly, GSH/GSSG level of 7721 was significantly reduced under NAC (ROS scavenger) intervention, which implies 7721 cells require a certain amount of ROS to maintain its GSH/GSSG level.

3.4. Endogenous ROS is involved in the regulation of 7721 cell telomerase activity

To investigate whether endogenous ROS was involved in the regulation of cancer cell telomerase activity, we examined the telomerase activity in sense/anti-sense MnSOD transfected 7721 cells. According to our results, MnSOD transfection in 7721 cells led to a decline of intracellular ROS, whereas antisense-MnSOD transfection increased ROS level, demonstrating the important role played by MnSOD in antioxidant system (Fig. 4B). Similarly, telomerase activity was inhibited in MnSOD transfected group, but was improved in anti-sense MnSOD transfected group (Fig. 4A). This phenomenon indicated that ROS indeed participate in telomerase activity regulation.

3.5. Akt signaling pathway is involved in the regulation of 7721 telomerase activity

Akt signaling pathway regulates tumor development and survival. Phosphorylated Akt mainly participate in the anti-apoptosis pathway, prevent PTEN-mediated cell apoptosis and keep cancer cell alive [18]. Our previous study showed that ROS is involved in Akt activation. We speculated that ROS may regulate telomerase through Akt pathway [19]. As we expected, NAC intervention significantly reduced Akt phosphorylation in 7721 cells (Fig. 5A). Correspondingly, H2O2 intervention increased Akt phosphorylation level in 7721, which was decreased under NAC intervention (Supplementary Fig. 2). These results confirmed that ROS indeed activated the Akt. Either Akt phosphorylation inhibitor, LY294002, or NAC could inhibit 7721 telomerase activity (Fig. 5B). These results suggest that ROS and Akt are both involved in upregulation of telomerase, and likely ROS is the upstream regulator that increases 7721 telomerase activity through activating Akt.

4. Discussion

Severe oxidative stress and aberrant redox control of proliferation are commonly found in tumors [20], and tumor cells apparently have developed adaptation to excessive ROS and even benefit from it. It has been reported that ROS is essential for insulin secretion, however paradoxically, an excess of ROS will kill beta cells [21]. It is proposed that the effects of ROS on cells may be presented in the form of a bell-shaped curve, where small amounts of ROS are signaling and very beneficial, but large amounts will cause cell damage.

It is evident that an increment in H_2O_2 concentration will lead to an elevation in cellular ROS levels. The increment in NAC, also known as ROS scavenger, may somewhat indirectly indicate a decline in cellular ROS. In contrast to the accelerated proliferation of L02 under moderate NAC treatment, 7721 required a little more oxidative stress generated by H_2O_2 to achieve its highest viability (Fig. 1A). We refer to the redox state when cell viability attains its "maximum" as "the redox homeostasis threshold". Previous studies have suggested that excessive antioxidant application will

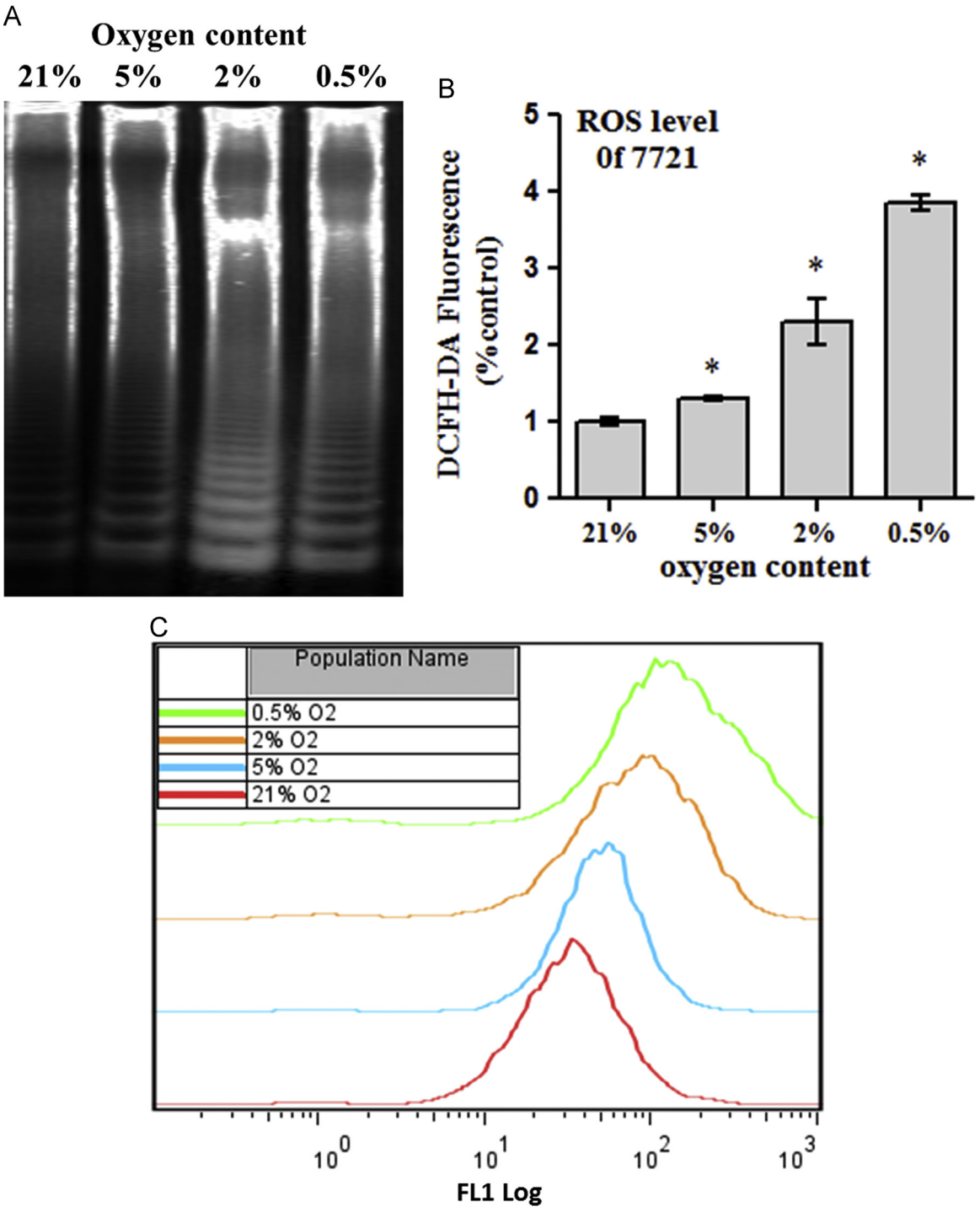

Fig. 2. Telomerase activity and ROS level of 7721 cells under different hypoxic conditions. (A) Telomerase activity of 7721 cells under different hypoxic conditions. Cells were cultured in a regular incubator under 21% O_2 condition or a hypoxia incubator under 5%, 2% or 0.5% O_2 condition for 24 h. Then the cells were collected in lysis buffer and telomerase activity was assayed. The brighter the strip is, the higher the telomerase activity is in the cell sample tested. (B) Intracellular ROS production in 7721 cells after incubated under 21%, 5%, 2%, 0.5% O_2 condition for 24 h. Cells were collected in PBS and staining with DCF-DA before the intracellular ROS was determination by Flow cytometry. Results shown represent mean ± SD, $n=3$, $^*p < 0.05$ vs. control group. (C) Flow-cytometry image of 7721 cells cultured in 21% (red line), 5% (blue line), 2% (orange line) and 0.5% (green line) O_2 condition for 24 h, stained with DCF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

induce cell apoptosis and leading to a significant increase in ROS production. Since the NAC concentration adopted in our model were generally below IC_{50}, and our results showed that such NAC concentrations significantly inhibited intracellular ROS production (Supplementary Fig. 1), we considered that the decline in cell viability of L02 and 7721 at higher NAC concentration was not due

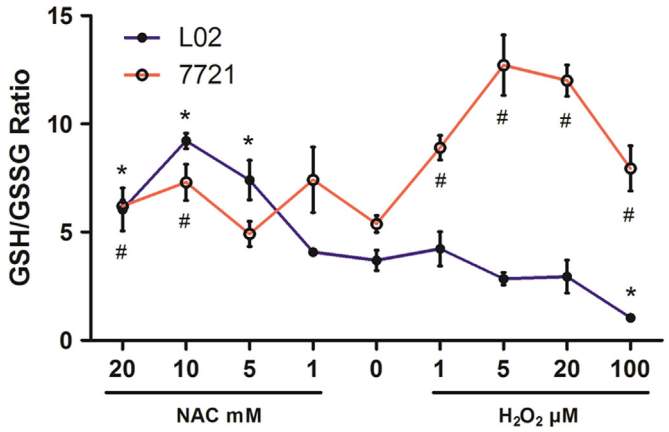

Fig. 3. GSH/GSSG ratios in L02 and 7721 cells. L02 (A) and 7721 cells (B) cultured in 1, 5, 20 μM H_2O_2 or 1, 5, 10 mM NAC for 24 h. GSH was assayed using OPC in protein-free extracts after GSSG reduction by DTT. Data are mean ± SD for cells from at least three independent experiments, $^*p < 0.05$ vs. L02 control group, $\#p < 0.05$ vs. 7721 control group.

to the excessive dose of NAC induced cell apoptosis, but instead attributable to the lack of ROS. Preference towards NAC or H_2O_2 implied that an essentially different redox microenvironment was required for cell proliferation between L02 and 7721 cells. The redox homeostasis threshold value for 7721 cells indicated 7721 cells were more prone to be in an oxidative status as compared to L02 cells. The results suggest that 7721 cells hold a higher redox homeostasis threshold than L02 cells

Telomerase variation tendency of L02 and 7721 cells (Fig. 1B) was in accord with the bell-shaped cell viability curve, indicating that the optimal extracellular redox state for the highest telomerase activity in 7721 and L02 was different, and 7721 telomerase was inclined to favor an oxidizing microenvironment while L02 telomerase preferred a reducing microenvironment. The respective telomerase activity of L02 and 7721 cells reached peak under their most appropriate redox state. This provided a theoretical basis for the inhibitory effect of NAC on cancer cells. Since tumor cells "prefer" a certain degree of oxidative microenvironment, whereas anti-oxidants cause tumor cells to thrive in a relatively reductive microenvironment, this reduces the redox state level below their most appropriate redox state i.e. the redox homeostasis threshold, which is not conducive to the maximum level of telomerase activation, thereby inhibiting cell viability. However, for normal cells, they are biased towards a reductive environment to achieve their redox homeostasis status, therefore NAC treatment could make the redox state closer to the threshold of the redox homeostasis through scavenging excessive ROS, which serves to stimulate telomerase activity and increasing cell viability. Thus NAC selectively inhibit cancer cells' telomerase activity through decreasing ROS level below the redox homeostasis threshold, but improve normal cells' telomerase activity by lowering ROS level to achieve their optimal redox state (Fig. 6).

The variation tendency of telomerase activity under hypoxia once again proves the existence of redox homeostasis threshold and cancer cells have more preference for a moderate oxidative microenvironment. Thus, it seems to be reasonable that NAC intervention cause a decline in cancer cell telomerase activity.

Glutathione system is considered to be the cells' first line defense against extracellular ROS. Moderate H_2O_2 intervention increased the GSH/GSSG of both L02 and 7721, but with increment of H_2O_2 concentration, GSH/GSSG ratio of L02 declines faster compared to that of 7721 (Fig. 3). This suggests that an appropriate amount of H_2O_2 can mobilize cell's antioxidant capacity, and 7721 cells have a greater demand for H_2O_2 to maximize their antioxidant ability. NAC is not only an ROS scavenger, but also one of precursors of glutathione synthesis. Therefore, besides eliminating excessive ROS, NAC could affect glutathione system and increase GSH content theoretically. However, GSH/GSSG level of 7721 was significantly reduced under NAC intervention, which implies that even though NAC could supplement cell glutathione pool, its ROS scavenger function was overwhelming. This proves that 7721 cells require a certain amount of ROS to maintain its GSH/GSSG level.

It was reported that moderate ROS could mobilize antioxidant system and up-regulate cell antioxidant protection [22], which will ultimately lead to accumulation of reducing substances (for instance reduced glutathione) within the cell. MnSOD is one of the most vital members of antioxidant system which is located in mitochondria [23], and rapidly converts super oxide anion to H_2O_2. Through a series of subsequent reactions, H_2O_2 is eventually decomposed into H_2O. MnSOD transfection accelerates ROS conversion, makes more super oxide anion transform into H_2O_2, which can be further decomposed to harmless water by other antioxidant enzymes including catalase, an abundant and most powerful one. Therefore sense-MnSOD transfection resulted in an endogenous decreased cellular ROS level and antisens-MnSOD transfection led to an endogenous increased cellular ROS level. Our results suggests that endogenous ROS is involved in the regulation of 7721 cell telomerase activity.

We have previously demonstrated that Akt phosphorylation level is decreased in MnSOD transfected 7721 cells, but is elevated in anti-sense MnSOD transfected ones [19,24]. Furthermore, GSH have been reported to activate PI3K/Akt signaling and inhibit the activation of FOXO3 [25]. Our results confirmed that ROS can activate Akt, and demonstrated that ROS-induced telomerase activity may be correlated with Akt activation. The Akt pathway has been shown to activate telomerase either by phosphorylation of the hTERT subunit [26] or by both post-translational and transcriptional mechanisms [27,28]. Therefore, ROS may regulate telomerase activity through Akt pathway.

5. Conclusion

Our results indicate that the difference of the redox homeostasis thresholds might be the main reason why telomerase activities in normal cell and cancer cell are different under NAC intervention. A much higher level of ROS is required for cancer cells' growth and proliferation, which in turn results in that tumor cells are more inclined to the oxidative redox homeostasis threshold. NAC may remove too much ROS in tumor cells than it is supposed to, and this affects the normal signaling transduction function mediated by ROS such as Akt pathway, which in turn affect telomerase activation. Our results disclose the mechanism by which antioxidants exerts different effects on telomerase activity of normal and cancer cells, and suggests that optimal doses of antioxidants should be used against the actual redox level in vivo. This provides a theoretical basis for application strategy of antioxidants. Findings of the present study may provide insights into novel approaches for treatment cancer.

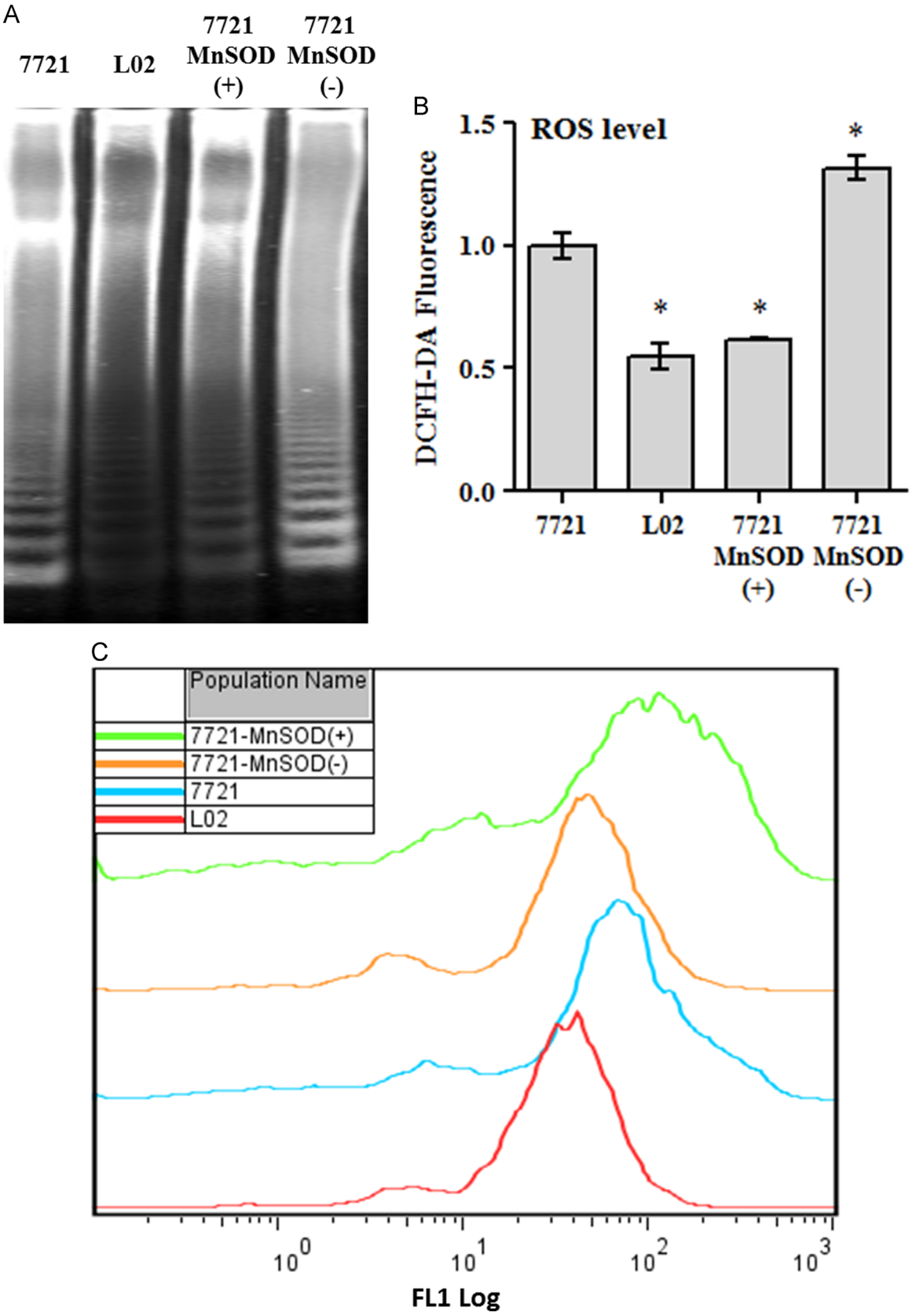

Fig. 4. Telomerase activity and ROS production in different cells. (A) Telomerase activity of different cells. The brighter the strip is, the higher the telomerase activity is in the cell sample tested. (B) Intracellular ROS production in different cells. Cells were collected in PBS and staining with DCF-DA before the intracellular ROS was determination by Flow cytometry. Results shown represent mean ± SD, $n=3$, $^{*}p<0.05$ vs. control group. (C) Flow-cytometry image of L02 (red line), 7721 cells transfected with empty plasmids (blue line) and 7721 cells transfected with sense (orange line) or antisense (green line) MnSOD plasmids, stained with DCF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

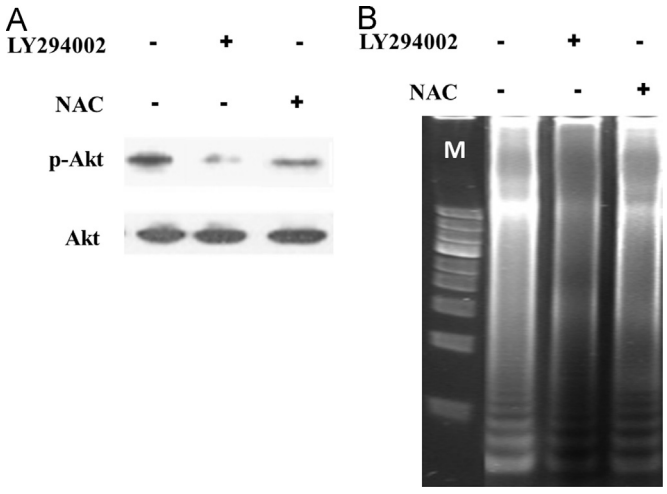

Fig. 5. Akt is involved in regulating telomerase activity. (A) Western blot analysis of phosphorylated-Akt and Akt in 7721 cells under intervention of NAC (5 mM) or Akt phosphorylation inhibitor LY294002 (20 μM) for 24 h. (B) Telomerase activity of 7721 cells under treatment of NAC (5 mM) and Akt phosphorylation inhibitor LY294002 (20 μM) for 24 h.

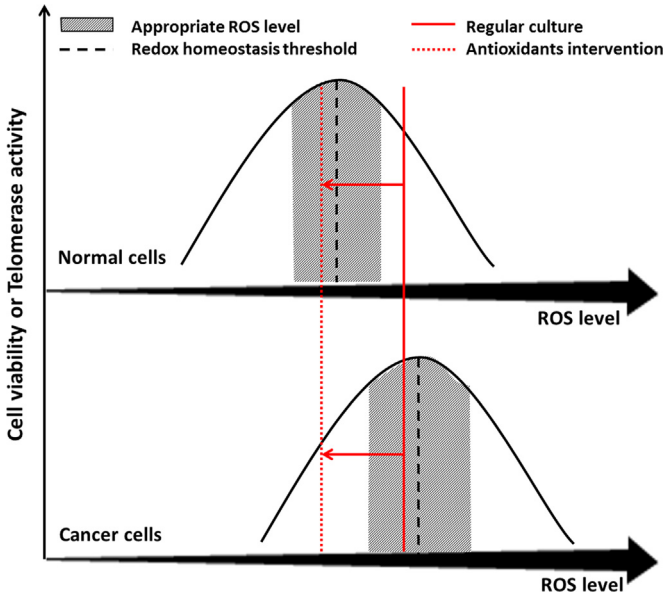

Fig. 6. Cancer cells hold a higher redox homeostasis threshold than normal cells. Under regular culture condition, the ROS level was too high for normal cells but slightly insufficient for cancer cells. NAC intervention makes the redox state closer to the normal cell's redox homeostasis threshold through scavenging excessive ROS, which serves to stimulate telomerase activity and increasing cell viability. But in cancer cells, NAC over-eliminate intracellular ROS and inhibit telomerase activity and cell viability.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.redox.2015.12.001.

References

[1] L.M. Colgin, R.R. Reddel, Telomere maintenance mechanisms and cellular immortalization, Curr. Opin. Genet. Dev. 9 (1999) 97–103.
[2] K. Furumoto, E. Inoue, N. Nagao, E. Hiyama, N. Miwa, Age-dependent telomere shortening is slowed down by enrichment of intracellular vitamin C via suppression of oxidative stress, Life Sci. 63 (1998) 935–948.
[3] J. Feng, W.D. Funk, S.S. Wang, S.L. Weinrich, A.A. Avilion, C.P. Chiu, The RNA component of human telomerase, Science 269 (1995) 1236–1241.
[4] T.M. Nakamura, G.B. Morin, K.B. Chapman, S.L. Weinrich, W.H. Andrews, J. Lingner, Telomerase catalytic subunit homologs from fission yeast and human, Science 277 (1997) 955–959.
[5] D.B. Hall, R.E. Holmlin, J.K. Barton, Oxidative DNA damage through long-range electron transfer, Nature 382 (1996) 731–735.
[6] K. Yudoh, V. Nguyen, H. Nakamura, K. Hongo-Masuko, T. Kato, K. Nishioka, Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function, Arthritis Res. Ther. 7 (2005) R380–R391.
[7] G. Kashino, S. Kodama, Y. Nakayama, K. Suzuki, K. Fukase, M. Goto, Relief of oxidative stress by ascorbic acid delays cellular senescence of normal human and Werner syndrome fibroblast cells, Free Radic. Biol. Med. 35 (2003) 438–443.
[8] M. Nasiri, N. Zarghami, K.N. Koshki, M. Mollazadeh, M.P. Moghaddam, M. R. Yamchi, Curcumin and silibinin inhibit telomerase expression in T47D human breast cancer cells, Asian Pac. J. Cancer Prev. 14 (2013) 3449–3453.
[9] S.K. Noureini, M. Wink, Antiproliferative effects of crocin in HepG2 cells by telomerase inhibition and hTERT down-regulation, Asian Pac. J. Cancer Prev. 13 (2012) 2305–2309.
[10] S.A. Sheweita, B.Y. Sheikh, Can dietary antioxidants reduce the incidence of brain tumors? Curr. Drug. Metab. 12 (2011) 587–593.
[11] D. Sadava, E. Whitlock, S.E. Kane, The green tea polyphenol, epigallocatechin-3-gallate inhibits telomerase and induces apoptosis in drug-resistant lung cancer cells, Biochem. Biophys. Res. Commun. 360 (2007) 233–237.
[12] Y. Bermudez, S. Ahmadi, N.E. Lowell, P.A. Kruk, Vitamin E suppresses telomerase activity in ovarian cancer cells, Cancer Detect. Prev. 31 (2007) 119–128.
[13] C.R. Reczek, N.S. Chandel, ROS-dependent signal transduction, Curr. Opin. Cell Biol. 33 (2015) 8–13.
[14] P.J. Hissin, R. Hilf, A fluorometric method for determination of oxidized and reduced glutathione in tissues, Anal. Biochem. 74 (1976) 214–226.
[15] N.W. Kim, M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L. Ho, Specific association of human telomerase activity with immortal cells and cancer, Science 266 (1994) 2011–2015.
[16] S.L. Liu, X. Lin, D.Y. Shi, J. Cheng, C.Q. Wu, Y.D. Zhang, Reactive oxygen species stimulated human hepatoma cell proliferation via cross-talk between PI3-K/PKB and JNK signaling pathways, Arch. Biochem. Biophys. 406 (2002) 173–182.
[17] N.C. Denko, Hypoxia, HIF1 and glucose metabolism in the solid tumour, Nat. Rev. Cancer 8 (2008) 705–713.
[18] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer, Nat. Rev. Cancer 2 (2002) 489–501.
[19] S. Dong-Yun, D. Yu-Ru, L. Shan-Lin, Z. Ya-Dong, W. Lian, Redox stress regulates cell proliferation and apoptosis of human hepatoma through Akt protein phosphorylation, FEBS Lett. 542 (2003) 60–64.
[20] J. Chiu, I.W. Dawes, Redox control of cell proliferation, Trends Cell Biol. 22 (2012) 592–601.
[21] B.E. Corkey, Banting lecture 2011: hyperinsulinemia: cause or consequence? Diabetes 61 (2012) 4–13.
[22] J.D. Hayes, M. McMahon, The double-edged sword of Nrf2: subversion of redox homeostasis during the evolution of cancer, Mol. Cell. 21 (2006) 732–734.
[23] G.H. Wong, J.H. Elwell, L.W. Oberley, D.V. Goeddel, Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor, Cell 58 (1989) 923–931.
[24] D.Y. Shi, F.Z. Xie, C. Zhai, J.S. Stern, Y. Liu, S.L. Liu, The role of cellular oxidative stress in regulating glycolysis energy metabolism in hepatoma cells, Mol. Cancer. 8 (2009) 32.
[25] J. Song, J. Park, Y. Oh, J.E. Lee, Glutathione suppresses cerebral infarct volume and cell death after ischemic injury: involvement of FOXO3 inactivation and Bcl2 expression, Oxid. Med. Cell. Longev. 2015 (2015) 426069.
[26] S.S. Kang, T. Kwon, D.Y. Kwon, S.I. Do, Akt protein kinase enhances human telomerase activity through phosphorylation of telomerase reverse transcriptase subunit, J. Biol. Chem. 274 (1999) 13085–13090.
[27] C. Zhou, V.L. Bae-Jump, Y.E. Whang, P.A. Gehrig, J.F. Boggess, The PTEN tumor suppressor inhibits telomerase activity in endometrial cancer cells by decreasing hTERT mRNA levels, Gynecol. Oncol. 101 (2006) 305–310.
[28] O. Uziel, E. Fenig, J. Nordenberg, E. Beery, H. Reshef, Sandbank, J Imatinib mesylate (Gleevec) downregulates telomerase activity and inhibits proliferation in telomerase-expressing cell lines, Br. J. Cancer 92 (2005) 1881–1891.