WWJMRD 2022; 8(03): 92-99
www.wwjmrd.com
International Journal
Peer Reviewed Journal
Refereed Journal
Indexed Journal
Impact Factor SJIF 2017: 5.182 2018: 5.51, (ISI) 2020-2021: 1.361
E-ISSN: 2454-6615
DOI: 10.17605/OSF.IO/NWG5F

Rupanwita Sarkar
Department of Zoology,
Dhruba Chand Halder College,
Dakshin Barasat, West Bengal,
India.

Correspondence:
Rupanwita Sarkar
Department of Zoology,
Dhruba Chand Halder College,
Dakshin Barasat, West Bengal,
India.

Effect of arsenic on telomerase and apoptosis in human keratinocytes and leukemia cells and epidermoid tumor cell line *invitro*

Rupanwita Sarkar

Abstract
Telomeres at the chromosome ends in eukaryotic organisms is important for the maintenance of genome stability. Telomeres are noncoding, hexanucleotide repetitive sequences. It is generally active in cell-types with high replication rates, including germ and immune cells. Telomeres are critical in maintaining chromosome. Arsenic, a human carcinogen is known for it's clastogenicity. About 80-90% of various cancer cells have detectable telomerase activity, so it is a potential anticancer target. Telomerase reverse transcriptase (TERT) is a key component of telomerase. The expression of TERT is a rate-limiting factor. The mammalian protein TERT (hTRT/hEST2/TP2/TCS1) contain reverse transcriptase motifs and is associated with telomerase activity. Low concentration (0.1-1µMm HaCaT and 0.1-0.5 µMin HL-60) of arsenite increased telomerase activity, maintained telomerase length. High Concentration (>1-40µM) of arsenite decreased telomerase activity, telomere length. 5,5-dimethyl-1 pyrroline-N-Oxide (DMPO) was effective in protecting the arsenic-included telomere attrition and apoptosis. The carcinogenic effects of arsenic maybe partly attributed to increase in telomerase activity and leading to telomeric DNA attrition.

Keywords: Telomerase, TERT, DMPO, Telomere, Proliferation, Cancer.

1. Introduction
Arsenic is an impotant environmental concern worldwide because millions of people are at risk of drinking water contaminated by this metal chappell *etal*.(1997). In West Bengal, India, ground water of 9 districts in contaminated with arsenic and concentration ranging from 52-1055 µg/l, much higher limit. Epidemiological data show that chronic arsenic exposure of human to inorganic arsenic is associated with peripheral neuropathy and a variety of cancers, particularly of the skin, lung, bladder, and liver Taylor *etal.* (1989). Arsenicals also have been reported to induce DNA and chromosome damage. DNA Feng *et al.* (2001). Arsenic exerts it's toxicity by generating reactive oxygen species (ROS) Liu *et al.* (2001). Telomeres consist of G-rich sequence that cap the ends of chromosomes protecting them from fusion. The telomerase RNA template (TR) cooperates with telomerase reverse transcriptase to form active telomerase, Blackburn. (2002). Telomere shortening and reactivation of telomerase and known to promote carcinogenesis in p53 mutant backgrounds, Outton et. al. (2000). Human cells are more sensitive to arsenite Lee et. al. (1989). The expression of human telomerase reverse transcriptase (hTERT) is a rate limiting step for telomerase activity. Telomerase is necessary for the sustained growth of most human tumors., Bouffler et al. (2001). Telomerase activity has been detected, Shay et al. (2001). Regulation of telomerase activity is critical in maintaining stability of chromosome. Arsenic is known for it's clastogenicity in human cells. Chou et al., show that arsenic inhibits telomerase transcription leading to chromosomal end lesions *invitro*, chou et. al. (2001). Human epidermal Keratinocytes (HaCaT) are primary targets of arsenic and is associated with chronic exposure to arsenic. Promyelocytic leukemia (HL-60) cells are also used to investigate the effects of arsenic. Arsenic trioxide has been used as an anticancer agent for acute promyelocytic leukemia cases.

2. Cell lines

HaCaT and HL-60 are the two main cell lines of the effects of arsenic. The A431 line is an epidermoid tumor cell line (derived from an 85-year-old patient), whereas 293 is an embryonic kidney cell line that was originally immortalized in culture with SV40T antigen. Both cell lines exhibit a transformed phenotype. These cells were first transfected with a *Drosophila* ecdysone receptor then with the mutant or wild type hTERTs that were placed under the control of the ecdysone-inducible promoter element. Cells were cultured in RPMI 1640 medium with 10% fetal bovine serum and in a humidified atmosphere of 95% air and 5% CO_2 at 37°C (Zhang et al., 2003).

3. Cell treatment and viability

A stock solution of 1mM sodium arsenite (Sigma, Sent Louis, MO) in RPMI 1640 medium was prepared. After 24 hours of self-seeding sodium arsenite was added to the cultures to reach the final concentration of 0.1 to 40 μM. After 1,3 and 5 days of arsenite treatment, cell growth including cell viability and total cell number was determined by MTT assay, a cell proliferation kit (Roche Molecular Biochemicals, Indiana-polis, IN) and tryphan blue exclusion method. In MTT assays, the cells were plated in the 96 well plates (triplicate wells for each treatment) at a density of $1x10^4$ cells in 0.2 ml of medium/well and cell growth was measured at 560 nm absorbance and expressed as the percentage of controls (Zhang et al., 2003).

4. Cell apoptosis

The treated and cotrol cells were harvested, centrifuged washed in PBS buffer and resuspended in PBS buffer at 10^5/ml. The cells were stained for 15 minutes with 10 μM Hoechst 33342 (Ho342, Molecular probes, Eugen) and then with 10 μM propidium iodide (PI, Molecular probes, Eugen) for 15 min. The percentage of apoptotic cells were determined by HO342 staining, while damage to the plasma membrane indicating cell necrosis was detected by PI staining. The defining characteristic of apoptosis is the change in cell morphology, including cell shrinkage, nuclear condensation and then segmentation and division into apoptotic bodies, Vu *et al.,* (1998). From each treatment, at least 300 cells were counted and the percentage of apoptotic and necrotic cells were calculated (Zhang et al., 2003).

5. Reactive oxygen species and arsenic effects

In order to assess the role of reactive oxygen species (ROS) on cell growth, apoptosis and telomere length, a spin trap agent (5,5 dimethyl-1-pyrroline-N-oxide or DMPO from sigma) was added at the concentration of 2.5mM to the cell cultures treated with arsenite and controls for 3 days (for telomere array) and 5 days (for cell growth and apoptosis assays). Cell viability and apoptosis were assessed to determine the effects of DMPO on arsenic treated cells (Zhang et al., 2003).

6. Telomerase activity

Telomerase activity in cell-extracts was measured by the PCR-bared telomere repeat amplification protocol (TRAP) using TRAPeze Kit (Intergen, Gaithersburg, MD). Cells were washed in phosphate-buffered saline and homogenized in the CHAPS lysing buffer for 30 min on ice. Protein (50-100 ng) from each cell extract was analyzed in the TRAP reaction. The cell extracts were added directly to the TRAP reaction mixture containing dNTPS, TS primer ($6x10^5$ c.p.m), reverse primer mixture and Taq DNA polymerase. Extended telomerase products were amplified by two-step PCR (94°e, 30s and 60°c, 30s) for 27 cycles. The bands indicating telomerase activity were analyzed using the Lumi-Imager (Boehringer Mannheim Corporation, Indianapolis, IN) with LumiAnalyst software. Telomerase activity was quantified by measuring the signal of telomerase ladder bands and was calculated as the ratio to the internal standard and expressed as percent of the controls, *Villa et.al. (2003).* (Zhang et al., 2003).

7. Telomerase and apoptosis

In order to access the role of telomerase on arsenic-induced apoptosis, telomerase negative (SW13, SW480) and positive cells (HT 1080), HL-60 and HaCaT were treated with arsenic ranging 0 to 40 μM for 5 days and percent of apoptotic cells were determined (Zhang et al., 2003).

8. Arsenic effects on cell growth

The effects on arsenite on cell growth of HL-60 and HaCaT by MTT assays are shown in Figure-1. When cells were exposed to arsenite ranging from 0.1-40 μM for 1,3 and 5 days, cellular growth was increased at low doses showing maximal effect at 0.5μM whereas cell growth was significantly decreased at concentration ≥ 1μM in both cell lines. The increase in cell growth was significantly different from the controls ($p<0.05$) for 5 days treatment for both cell lines. Arsenic induced cell proliferation at low concentrations and inhibited cell growth at higher concentrations (Zhang et al., 2003).

9. Arsenic effects on apoptosis

In all the cell cultures treated with various concentrations of arsenite, the necrotic calls (showing PI and trypan blue positive staining cells) were all below 5%. Dose and time related apoptotic effects from arsenite exposure after 1, 3 and 5 days were observed in both cell lines (Figure 1). when the concentration of arsenite were <1μM induction of cell apoptosis by arsenite were <1μM, induction of cell apoptosis by arsenite was not significantly increased.The percentage of apoptotic cells was significantly increased ($P<0.01$ and $P<0.05$ for HL-60 and HaCaT respectively for 5 days treatment) when arsenite concentrations were higher than 1μM. HL-60 cells were more susceptible to apoptosis from arsenite treatment than HaCaT cells. Both cell lines show arsenic induced cell proliferation and apoptosis depending on the dose of arserite (Zhang et al., 2003).

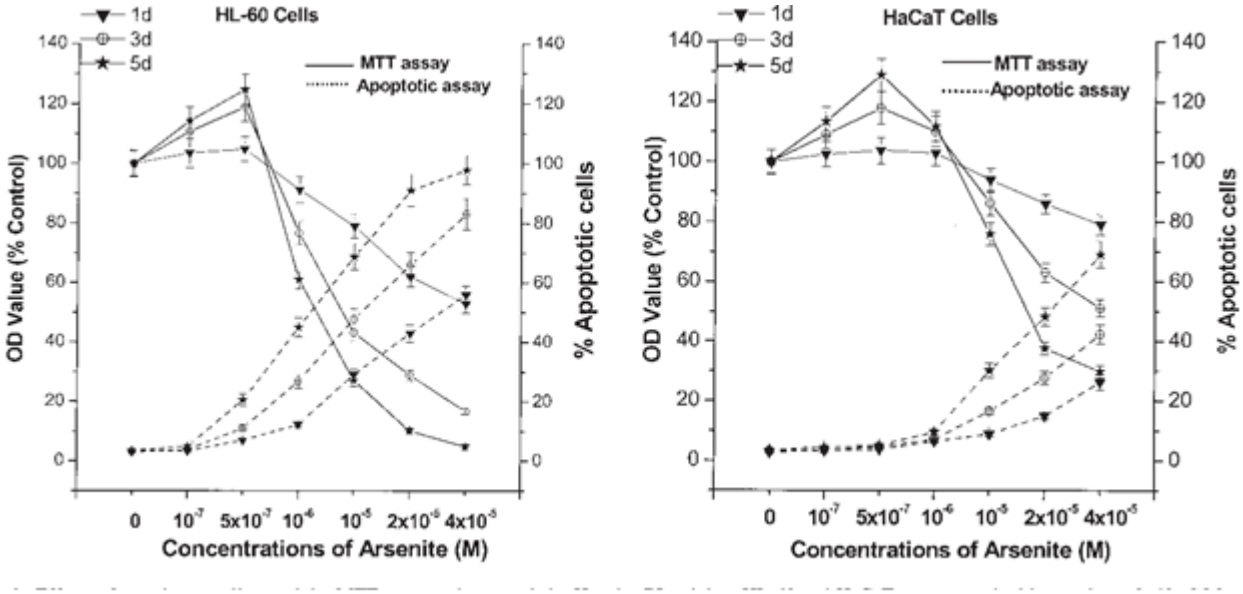


Fig. 1: Effects of arsenite on cell growth by MTT assay and apoptosis by Hoechst/PI staining, HL-60 and HaCaT were treated with arsenite at 0-40μM for 1-5 days. In MTT assays, data shown are cell viability measured by optical density (OD) at 360 nm and expressed as percent of controls. Apoptosis was expressed in the percent of apoptotic cells. All assays were conducted in three replicates for each treatment. Data shown are mean±SD from three independent experiments (ref., Zhang et al., 2003).

10. Effects of DMPO on arsenic-induced cell growth and apoptosis

The results of adding DMPO, a scavenger of ROS, to the arsenic-treated cultures significantly decreased the arsenic induced apoptosis in both cell lines ($P<0.01$ and $P<0.05$ for HL-60 and HaCaT, respectively) Shown in Figure 2. The cultures treated with 40μM arsenic showed a decrease of 34 and 40% respectively, in HL-60 and HaCaT in apoptosis by DMPO. DMPO showed no effects on the cell proliferation. No significant effects were observed on cell viability from DMPO treated alone. These results suggest that ROS may be involved in the arsenic-induced apoptosis but not involved in the arsenic-induced cell growth (Zhang et al., 2003).

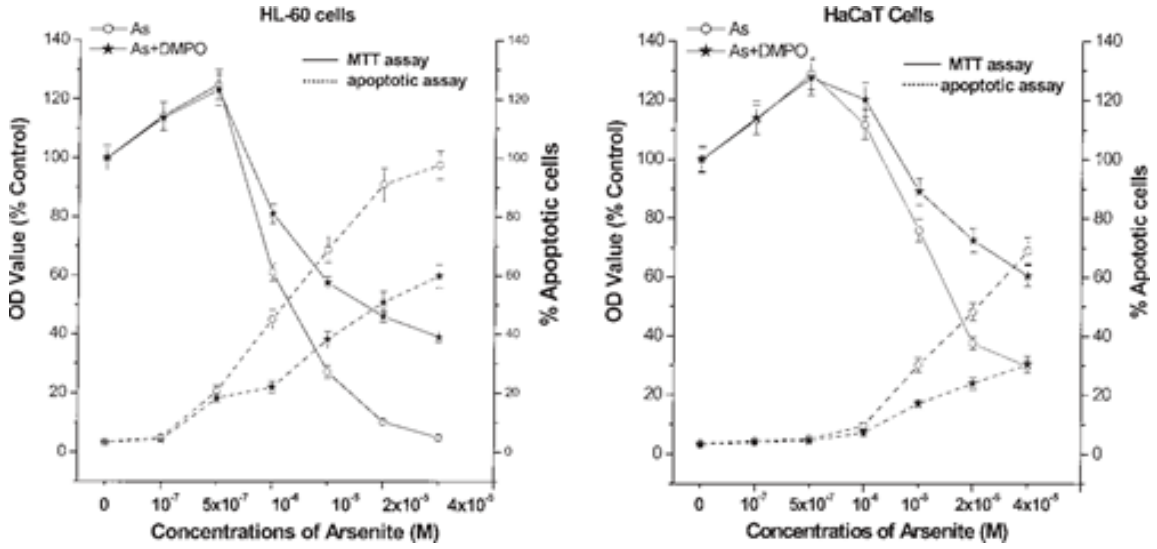


Fig. 2: Effects of DMPO on arsenite-induced cell growth and apoptosis in HL-40 and HaCaTcells. All cells were treated for 5 days with arsenite at 0-40μM or arsenite at the same concentration combined with 2.5mM DMPO. Cell viability and apoptotic cells were determined by MTT assay and Hoechst/PI staining assay, respectively. All assays were conducted in three replicates for each treatment. Data shown are mean ±SD from three independent experiments (ref., Zhang et al., 2003).

11. Arsenic effects on telomerase activity

The effects of arsenite on telomerase activity or hTERT protein-expression were shown in Figure 3. The control cells of HL-60 (a tumor cell line) and HaCaT(an immortal cell line) were positive in protein expression as expected. Protein expression of hTERT in both cell lines showed an increase at 0.5μM arsenite treatment and a decrease at 20μM as shown in Figure 3D, E, G and H.

The effects of arsenite on telomerase activity by TRAP assays were shown in Figure 4A and B (for quantification). At a low dose (0.5μM) telomerase activity was elevated 2-fold for HL-60 and 3fold for HaCaT whereas at high doses

(10 and 20μM) the activity was inhibited. There is a dose dependent decrease in telomerase activity following arsenite treatment at 10 and 20μM concentrations in HL-60 (Zhang et al., 2003).

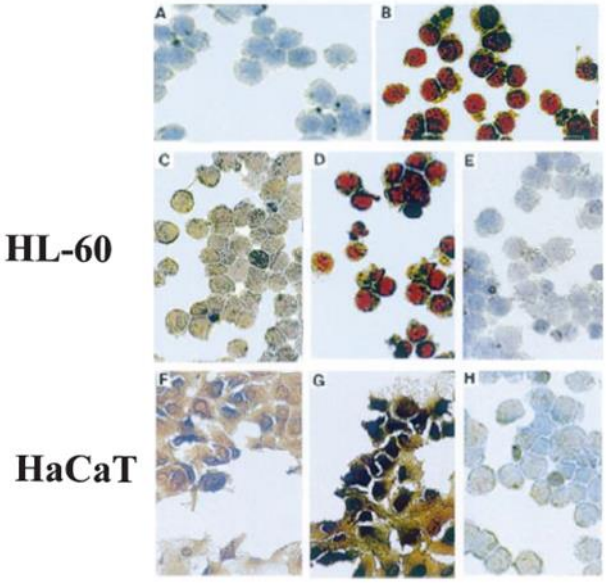


Fig. 3: hTERT protein expression by immunocytochemistry staining. (A) SW13 cells as the negative controls. (B) HT1080 cells as the positive controls. (C-E) HL-60 cells treated with arsenite at 0, 0.5 and 20μM, respectively, for 3 days. (F-H) HaCaT cells treated with arsenite at 0, 0.5 and 20μM, respectively, for 3 days. Arsenite significantly elevated hTERT protein expression at low dose (0.5μM) as shown in (D) and (G), whereas arsenite significantly inhibited hTERT protein expression at high dose (20μM) as shown in (E) and (H) in both cell lines (ref., Zhang et al., 2003).

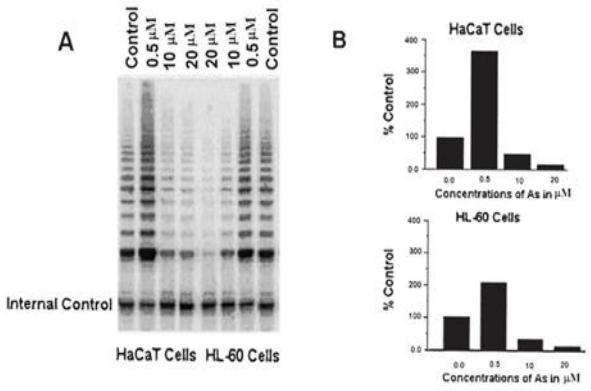


Fig. 4 Effects of arsenite on telomerase activity. (A) Telomerase activity by TRAPeze assay in HL-60 and HaCaT cells. Cells were treated with 0, 0.5, 10 and 20 μM of arsenite for 3 days and telomerase activity was determined. The experiment was repeated and the results were in agreement. (B) Quantification of telomerase activity from TRAPeze assay in (A). Telomerase activity was quantified by measuring the signal of telomerase ladder bands, and dividing by the intensity of the bands from the internal controls. The values were expressed as percent of the controls (ref., Zhang et al., 2003).

12. Telomerase and arsenic-induced apoptosis

The results comparing the telomerase negative and positive cell lines and HL-60 and HaCaT showed that the telomerase negative calls, SW480 and SW13 were more resistant to arsenic-induced apoptosis, whereas the telomerase positive cells (HT1080) were more sensitive to arsenic-induced apoptosis as shown in Figure 5. The apoptotic rate of HL-60 and HaCaT were also shown in Figure 5 to compare with these telomerase negative and positive cells. The results suggest that telomerase activity may be related to arsenic-induced apoptosis (Zhang et al., 2003).

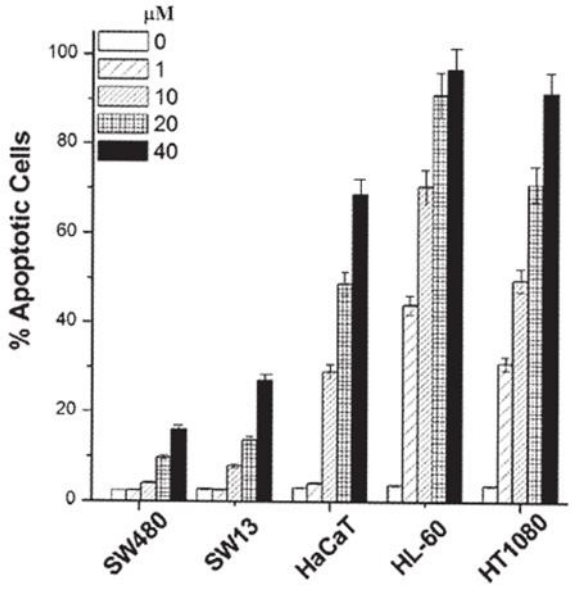
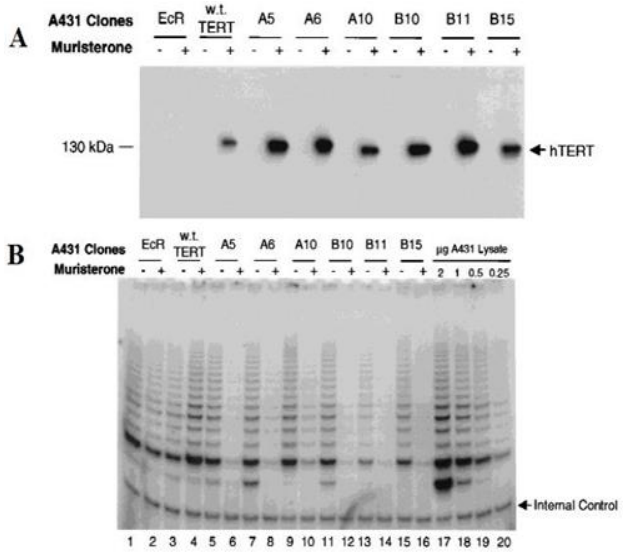


Fig. 5: Comparing telomerase-negative and -positive cells to investigate the effects of telomerase on arsenic-induced apoptosis. All cell lines were treated with arsenite 0-40μM for 5 days. Cell apoptosis was determined by Hoechst/PI staining assays. All assays were conducted in three replicates for each treatment and data shown are mean ± SD from three independent experiments (ref., Zhang et al., 2003).

13. Telomerase inhibition in A431 cells leads to rapid cell death

The A431 line is an epidermoid tumor cell line (derived from an 85-year-old patient). A431 cell line exhibiting the shortest average telomere length. Six isolated mutant clones and a wild type hTERT clone were all highly inducible as assayed by muristerone-dependant protein expression (Fig. 6A). The induction of dominant negative mutants by muristerone dramatically inhibited the telomerase activity in these cells, whereas the induction of the wild-type hTERT protein resulted in slightly increased telomerase activity (Fig.6B). Each clone was split into two populations and grown in the presence or absence of exogenously added muristerone. Several days after induction, all of the mutant cell lines exhibited morphological changes accompanied by the apparent death of the cells over a 5-10day period (Fig.6C) (Zhang et al., 2003).



Telomerase inhibition in human tumor cell lines

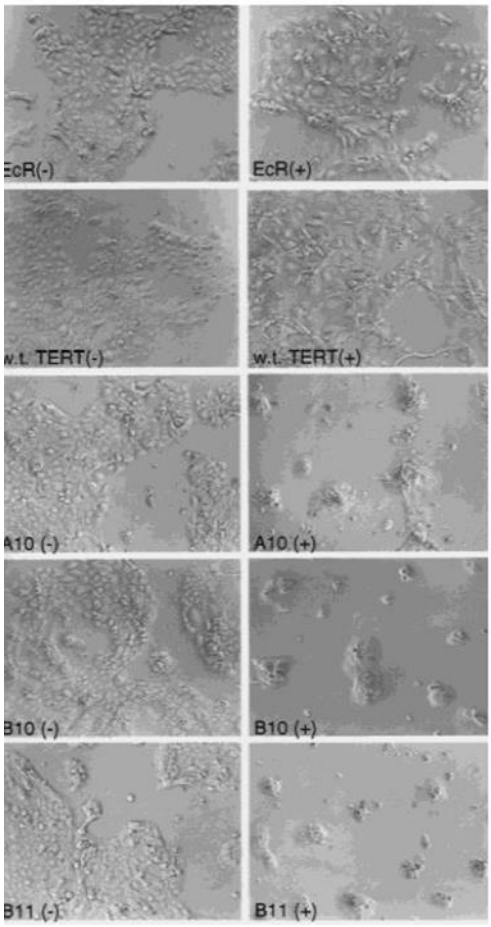


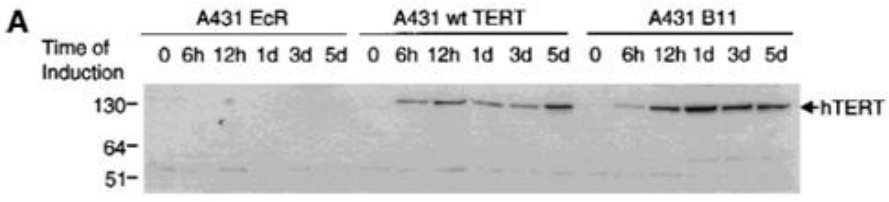
Fig. 6: (A) Muristerone induces the expression of dominant-negative TERT mutants. Cell extracts (15 µg/lane) from A431 stable clones with or without the induction of muristerone were detected by Western blotting. The hTERTs are labeled. The same antibody as in Fig. 1 was used. (B) Expression of dominant-negative TERT mutants inhibits telomerase activity in A431 cells. TRAP assay of the same lysate (1 µg) as in B was performed. (Lanes 17-20). The activity of serial dilution of parental A431 cell lysate. The internal control for PCR reaction is shown as labeled. (C) Morphology of A431 cell lines expressing control vector (EcR), wild-type TERT (D2), and mutant TERTs (A10, B10, B11) (ref., Zhang et al., 2020).

14. A431 cell death is apoptotic

A431EcR, wild-type and mutant TERT cell lines were grown over an 8-day period in the presence or absence of muristerone. Both wild-type and mutant TERT protein was observed by 6 hour post induction reaching steady-state levels by 24 hour (Fig.7A). The EcR wild-type and mutant cell lines had comparable growth rate when uninduced (Fig.7A). In contrast, growth curves from cells expressing the dominant negative mutants showed little-to-no increase in cell number in comparison with EcR and wild-type cells (Fig.7B). Suggesting that telomerase inhibition exerted a specific effect on tumor cell viability. The effect appeared to be caused by a decrease in the percentage of S-phase cells, no increase in the apoptotic rate was observed.

Cytological observation of the telomerase-inhibited cells at later time points suggested an appearance consistent with programmed cell death.

To determine whether the cells were undergoing apoptosis and to examine the time course of cell death, flow cytometry analysis of living cells double labelled with annexin V and propidium iodide (PI) over the eight-day induction period (Fig.7C). An early feature of apoptotic cells is the loss of plasma membrane asymmetry reflected by the exposure of phosphatidylserine (PS) from the inner leaflet to the outer leaflet Fadok et al., (1992). The high affinity PS-binding protein Annexin has been used widely to detect early-stage apoptotic cells Vermes et al., (1995). When combined with PI, which stains DNA of cells with permeable membrane, this approach allows a further distinction of early apoptotic (annexinV+/ PI -) and late apoptotic/necrotic (annexinV+/ PI +) cells. EcR and wild-type A431 cells showed an average of 3.4% of annexinV+/ PI + cells and 4.3% of annexinV+/ PI - cells. No increase of apoptotic cell death in these two cell lines could be detected by annexin V and PI. By day 8, mutant A431 had undergone massive apoptosis (Zhang et al., 2003).



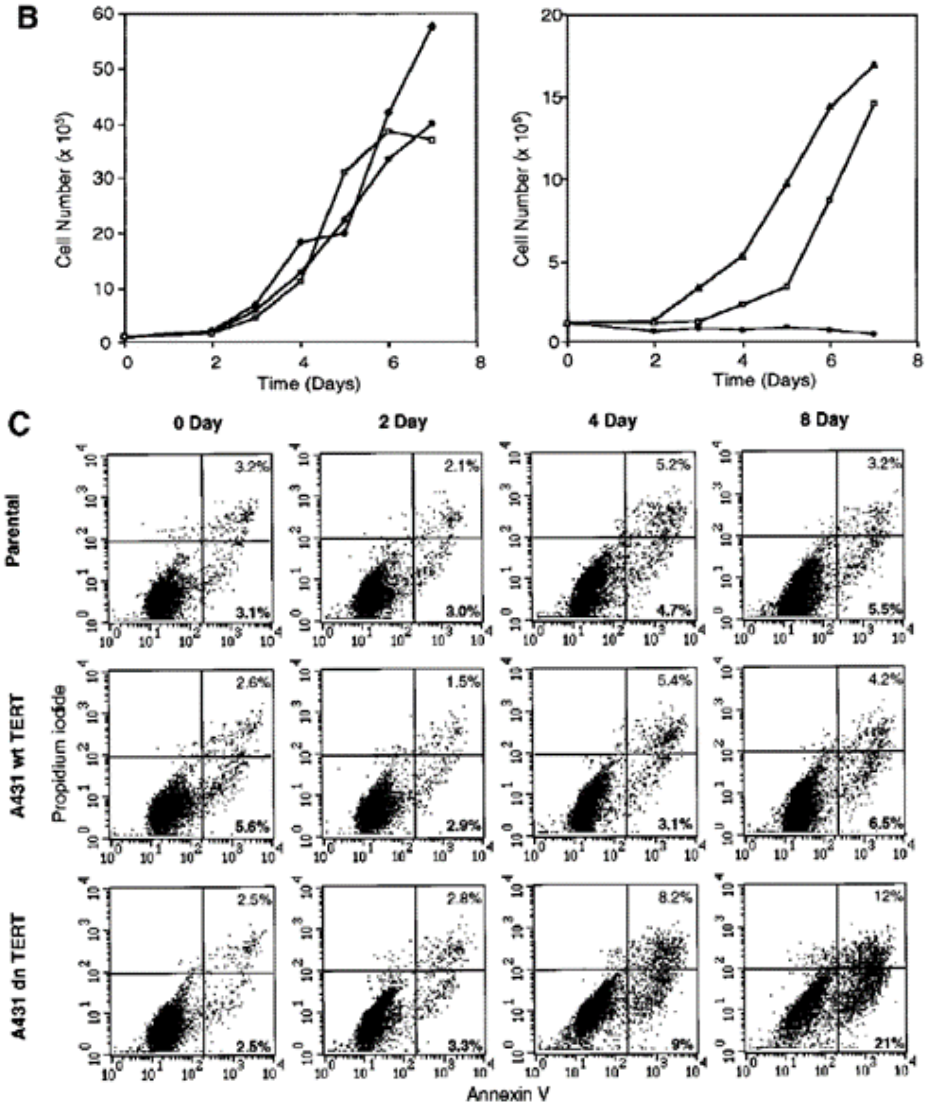


Fig. 7: (A) Kinetics of exogenous TERT expression. One set of cells per indicated day was lysed, and the total cell lysate (5μg/lane) was run on SDS-PAGE and Western blotted with the anti-TERT peptide antibody as in Fig. 1. (Arrow) The position ofhTERT.(B) Growth curve of A431 cells. Equal number of cells (1.2 × 105) from each cell line were plated into six-well plates in triplicate in the presence or absence of muristerone (1 μM) and allowed to grow for 7 dayswith medium being changed on day 4. Cell numbers were counted each day with a hemocytometer. Data shown are mean of the triplicate from one of three experiments all showing similar results. EcR (*Left*) Uninduced;w.t. TERT;B11. (*Right*) Induced; ECR; w.t. TERT; B11. (C) Flow cytometry analysis of cells double labeled with annexin V and PI. One set of cells from each cell line was harvested at indicated days. Cellswere then stained and analyzed as described in Material and Methods. (Ref, Zhanget al., 2020).

15. Apoptosis is telomere length dependent

The induction of apoptosis in A431 cells by inhibition of telomerase activity is dependent on the telomere length. Figure 8A shows the level and extent of TERT protein expression and inducibility for two mutants (A1, A2) and one wild-type TERT (D1) expressing cell lines. The expression of TERT proteins was induced to a higher level in A2 and wild-type lines, all three lines exhibit at the constitutive expression of TERT transgenes. The constitutive expression of dominant-negative TERT in A2 was sufficient to reduce endogenous telomerase activity in the uninduced cells (Fig. 8A) (Zhang et al., 2003).

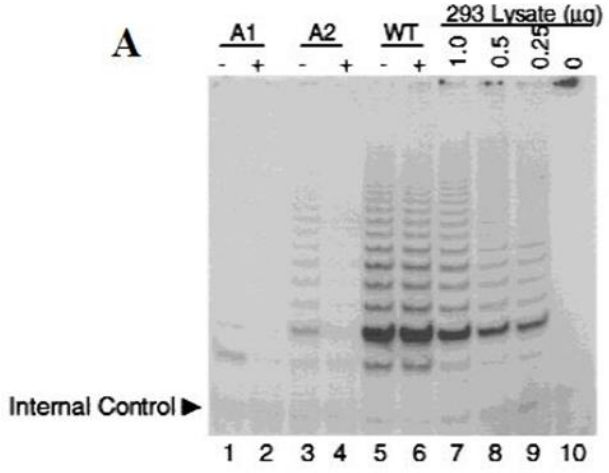


Fig. 8: (A) TRAP assay of the wild-type, A1, and A2 clones. (Lanes *7–10*) The serial dilution of nontransfected 293 lysate. One microgram of each lysate was assayed. (ref., Zhang et al., 2020)

Conclusion

Arsenite disrupts mitochondrial function and induces oxidative stress leading to telomere attrition. Arsenic may cause telomere attrition and chromosome fusion. chou et al.(2001) It has been demonstrated that the chromosome with the shortest telomere is the most unstable and most likely to fuse with another chromosome with a short telomere. Hemann et al., (2001). Telomere shortening may serve as an important checkpoint to limit the potential of human cells to proliferate Maser et al., (2002). Inhibition of telomerase activity suppresses the growth of human cancer cells and leads to chromosomal damage and apoptosis, Zaffaroni et al., (2002). Arsenite altered telomerase activity and telomere length in HL-60 and HaCaT cells. At low concentration (<1.0 μM) arsenite promoted telomerase activity and increased or at least maintained telomere length. Treatment with high concentration of arsenite (>1.0 μM) decreased telomerase activity and showed a rapid and dramatic loss of telomeric DNA, leading to cell apoptosis, chou et al, reported that arsenic trioxide at 0.75-1.0 μM inhibited telomerase transcription and resulted in chromosomal end lesion which promote either genomic instability toward carcinogenesis or cancer cell death in NB4 cells, chou et al., (2001).Telomere and telomerase play a dual role in tumorigenesis and replicate senescence. In Immortal cells the maintenance of telomerase and telomere can stabilize genomic instability and chromosomal abnormalities and thus permit unlimited cell proliferation whereas in non-immortal cells inhibition of telomerase activity and telomere shortening causes either cell apoptosis or promotion of chromosomal end-to-end fusion which may lead to initiation of carcinogenesis, Hackett et al., (2002). Telomerase activity and telomere length by arsenic may partly explain paradoxically carcinogenic and anticancer effects of arsenic. Telomere-negative cell lines are more resistant and telomerase play some role in the arsenic induced apoptosis.

Arsenic at high dose induced dramatic loss of telomeric DNA and showed a rapid increase in apoptosis in HL-60 and HaCaT cells. Liu et al. reported that arsenic induced osidative stress promotes telomere attrition and apoptotic cell death in mouse embrtos. Liu et al., (2003). HL-60 cells which are promyelocytic leukemia cells were more sensitive than the skin keratinocytes, HaCaT cells in decreasing telomerase activity and shortening of telomeres and induction of apoptotic effects by arsenite.

Telomerase activity appears to be required to maintain a minimum telomere length. A431 cells were maintained in DMEM supplemented with 10% fetal calf serum 100 U/ml of penicillin 100 μg/m of streptomycin, 400 μg/ml of zeocin and 700 μg/ml of G418. For induction of wild-type and mutant TERTs cells were seeded at a density of $1.25x10^4$ cells/cm^2, allowed to attach and grow overnight and change to the medium containing muristerone(1μM for A431 cells). The cells were then allowed to grow in this medium for a length of time depending on each individual experiment. For quantitative analysis of apoptosis living A431 cells were tryprinized, washed once in ice-cold PBS, and incubated with annexin-V-fluorescein PI (Boehringer Mannheim) in calcium-containing HEPES buffer; the cells were then immediately analyzed with a FACScan machine (Becton Dickinson).

For cell cycle analysis cells were fixed and stained by propidium iodide. The DNA content of each cell population was then analyzed by flow cytometry. DNA synthesis was measured by BrdU incorporation, protein concentration of the lysates was measured by Bradford assay (Bio-Rad). Telomerase activity was assayed by telomere repeat amplification protocol (TRAP) using the $TRAP_{ENE}$ telomerase detection kit (Oncor, Gaithersburg, MD), Zhang et al., (2020)

Acknowledgments

Author wishes to convey sincere thanks to principal, Dhruba Chand Halder College for his support. Mr. Debadyuti Paul is thankfully acknowledged for the illustrations in the review.

References

1. Chappell, W.,R. Beck, B., D. Brown, K.,G. Chaney, R. Cothern, R. Cothern, C.,R. Irgolic, K.,J. North, D.,W. Thornton, I, and Tsungas, T.,A. 1997. Inorganic arsenic: a need and an opportunity to improve risk assessment. Environmental Health perspectives. 105(10): 1060-1067.
2. Taylor, P., R. Qiao, Y., L. Schatzkin, A. Yao, S., X. Lubin, J. Mao, B., L. Rao, J., Y. McAdams, X., Z, and Li, J.,Y. 1989. Relation of arsenic exposure to lung cancer among tin miners in Yunnan province, China. British Journal of Industrial Medicine 46(12): 880-886.
3. Feng, Z. Xia, Y. Tian, D. Wu, K. Schmitt, M. Kwok, R and Mumford, JL, 2001. DNA damage in buccal epithelial cells from individuals chronically exposed to arsenic via drinking water in Inner Mongolia, China. Anticancer Research 21(IA): 51-58.
4. Liu, S., X. Athar, M. Lippai, I. Waldren, C. and Hei, T., K. 2001. Induction of oxyradicals by arsenic: Implication for mechanism of genotoxicity, proceedings of the National Academy of Sciences of the United States of America 98(4): 1643-1648.
5. Blackburn, E., H. 2000. Telomere states and cell fates. Nature 408(6808): 53-56.
6. Outlon, R. and Harrington; L. 2000. Telomeres, Telomerase and cancer: life on the edge of genomic stability. Current opinion in oncology 12(1): 74-81.
7. Bouffler, S., D. Blasco, M., A. Cox, R. and Smith, P., J. 2001. Telomeric sequences, radiation sensitivity and genomic instability. International Journal of Radiation Biology.77 (10): 995-1005.
8. Shay, J., WZou, Y. Hiyama, E and Wright, W., E. 2001. Telomerase and Cancer. Human Molecular Genetics. 10(7): 677-685.
9. Chou, W. Hawkins, A., L. Barrett, J., F. Griffin, C., A. and Dang, C., V. 2001. Arsenic inhibition of telomerase transcription leads to genetic instability. Journal of clinical investigation.108 (10): 1541-1547.
10. Vu, T., H. Shipley, J., M. Bergers, G. Bergers, J., E. Helms, J., A. Hanahan, D. Shapiro, S., D. Senior, R., M. and Werb, Z. 1998. MMP-9/ Gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell. 93(3): 411-422.
11. Villa; R. Folini, M. Porta, C., D. Valentini, A. Pennati, M. Daidone, M., G. and Zaffaroni, N. 2003. Inhibition of telomerase activity by geldanamycin and 17-allylamino 17-demethoxy geldanamycin in human melanoma cells, Carcinogenesis, 24(5): 851-859.
12. Fadok, V., A. Voelker, D., R. Campbell, P., A. Cohen; J., J. Bratlon, D., L. Henson, P., M. 1992. Exposure of

phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. Journal of Immunology. 148(7): 2207-2216.
13. Vermes, I. Haanen, C. Steffens-Nakken, H.Reutelingsperger C. 1995. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. Journal of Immunology. 184(1): 39-51.
14. Zhang, X. Mar, V. Zhou, W. Harrington. L. Robinson, M., O. 1999. Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. Genes and Development. 13(18): 2388-2399.
15. Hemann, M., T. Rudolph, K., L. Strong, M., A. DePinho, R., A. Chin, L. Greider, C., N. 2001. Telomere dysfunction triggers developmentally regulated germ cell apoptosis. Molecular Biology of the Cell. 12 (7): 2023-2030.
16. Tong-Cun Zhang, Michael T. Schmitt, Judy L. Mumford, 2003. Effects of arsenic on telomerase and telomeres in relation to cell proliferation and apoptosis in human keratinocytes and leukemia cells in vitro. Carcinogenesis. 11(24). 1811-1817.
17. Xiaoling Zhang, Vernon Mar, Wen Zhou, Lea Harrington, and Murray O. Robinson. Telomere shortening and apoptosis in telomerase-inhibited human tumor cells, 1999. Genes & Dev.13: 2388-2399.