Telomerase activity regulation is promoter methylation-sensitive

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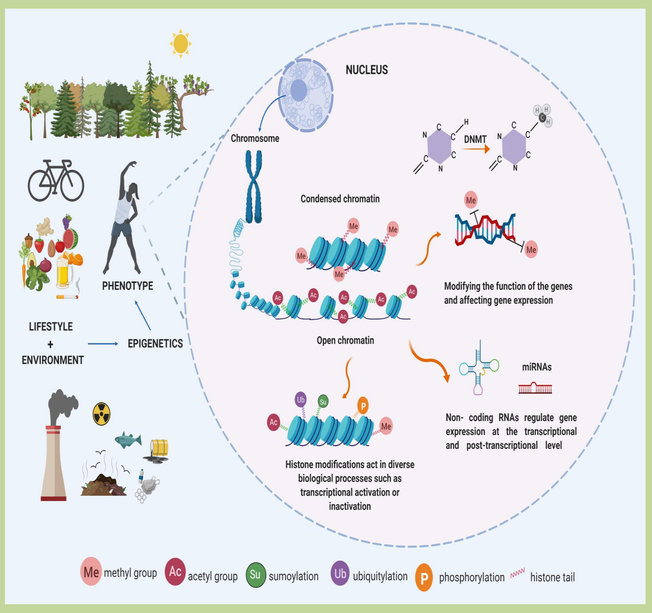
Introduction

Telomerase is responsible for telomere elongation where the telomerase reverse transcriptase gene (hTERT) encodes the catalytic subunit.

Understanding the role of epigenetic molecular mechanisms behind telomerase regulation holds important prospects for cancer. Promoter methylation has been suggested to be associated with epigenetic control of telomerase regulation which holds significant potential for therapy.

Objective : Here we sought to understand promoter methylation patterns in pluripotent stem, somatic, cancer cells and their role on telomerase activity.



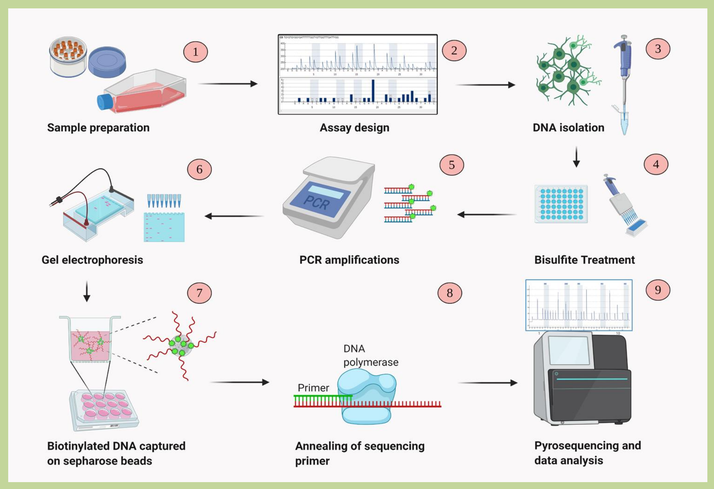
Methodology

Pluripotent stem cells were cultured in air oxygen standard incubator, 2%O2 controlled-oxygen workstation (2%WKS) and 2%O2-Pre-gassed media in a 2%O2 incubator (2%PG).

Telomerase activity was measured with TRAPeze®RT Telomerase Detection Kit (Millipore, USA).

Telomere length measured with Telomere Length Quantification qPCR Assay (Sciencell, USA).

TERT gene expression analysed using QPCR.



An overview of the steps involved in DNA methylation analysis using pyrosequencing

Results

hTERT gene expression and telomerase activity significantly decreased in all conditions during differentiation of pluripotent stem cells. Telomerase activity was significantly higher in physiologic oxygen vs. air cultured cells.

Significantly, hypermethylation of TERT promoter region during differentiation was associated with suppressed hTERT gene expression and telomerase enzyme activity. Methylation analysis results demonstrated a distinct methylation signature that distinguished somatic and pluripotent stem cells from cancer cells.

After Nanaomycin A (DNMT3B enzyme inhibitor) treatment, all cancer cells displayed significantly reduced enzyme activity. Also reduced DNMT3B gene expression noted after inhibitor treatment. Most importantly DNMT3B inhibitor reduced hTERT promoter methylation in all cancer cells. DNMT3B inhibitor increased surprisingly hTERT expression and telomerase activity.

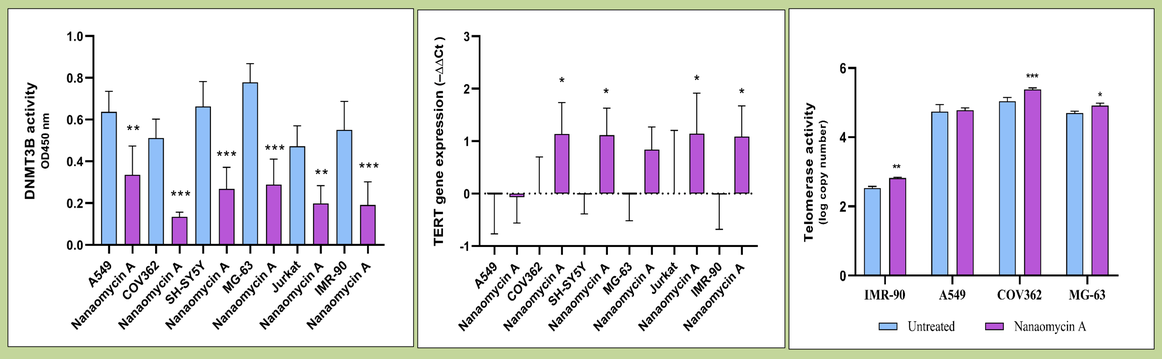
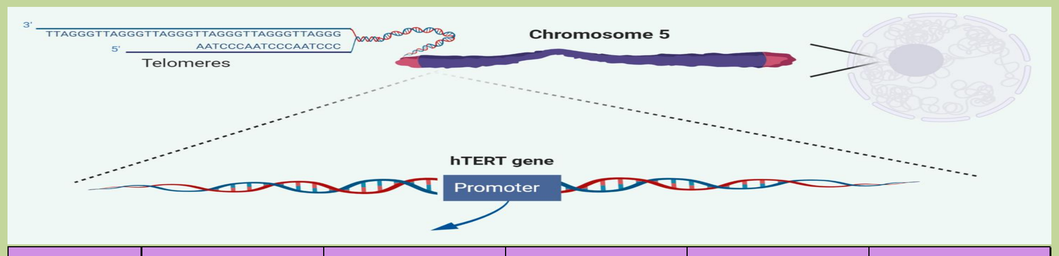


Figure 1. DNMT3B enzyme activity detection via colorimetric assay and telomerase activity detection with Real-time PCR. Cancer cells were obtained from 2%WKS. Data are represented as mean ± standard deviation (SD), *P<0.05, **P<0.01, ***P<0.001.



Cell Type	(-67 -106)	(-122, -171)	(-315 -348)	(-674, -717)	(-1456, -1495)
A549	4.6	7.0	81.3	68.6	86.9
Nanaomycin A	5.2	6.5	45.1	52.9	48.2
COV362	10.3	23.7	86.2	90.2	75.4
Nanaomycin A	9.4	19.5	73.9	89.4	73.1
SH-SY5Y	32.7	27.7	22.5	40.6	53.9
Nanaomycin A	14.5	15.0	21.3	29.7	58.8
MG-63	24.5	42.7	56.1	91.3	82.1
Nanaomycin A	14.3	23.0	36.6	77.4	68.9
Jurkat	69.7	82.4	87.9	73.3	73.3
Nanaomycin A	42.8	71.7	75.8	68.1	60.6
IMR-90	11.2	10.8	7.3	42.1	83.2
Nanaomycin A	8.7	6.1	6.8	41.4	76.6
SHEF2 21%	5.7	9.3	10.5	47.4	93.1
DF SHEF2 10	6.1	9.8	16.3	55.1	93.5
DF SHEF2 20	6.4	10.8	19.8	58.3	92.3
DF SHEF2 40	7.1	10.2	19.4	59.2	91.9
SHEF2 WKS	3.9	6.5	8.3	39.0	93.0
DF SHEF2 10	6.2	8.4	13.2	53.2	92.9
DF SHEF2 20	6.7	9.6	14.2	56.7	93.5
DF SHEF2 40	6.7	10.6	17.4	56.8	92.3
SHEF1 21%	5.7	10.5	4.0	42.5	86.3
DF SHEF1 10	6.0	10.0	7.7	51.9	86.2
DF SHEF1 20	8.8	11.6	10.7	62.0	84.6
DF SHEF1 40	8.9	16.4	11.8	55.9	84.9
SHEF1 WKS	3.9	6.0	3.3	30.6	85.7
DF SHEF1 10	4.9	7.9	7.3	51.5	86.2
DF SHEF1 20	5.1	10.3	11.5	56.4	84.5
DF SHEF1 40	7.9	16.2	9.5	50.8	85.9

Table 1. hTERT promoter methylation patern in five different regions relative to the transcription start site in pluripotent stem cells, cancer and somatic cells

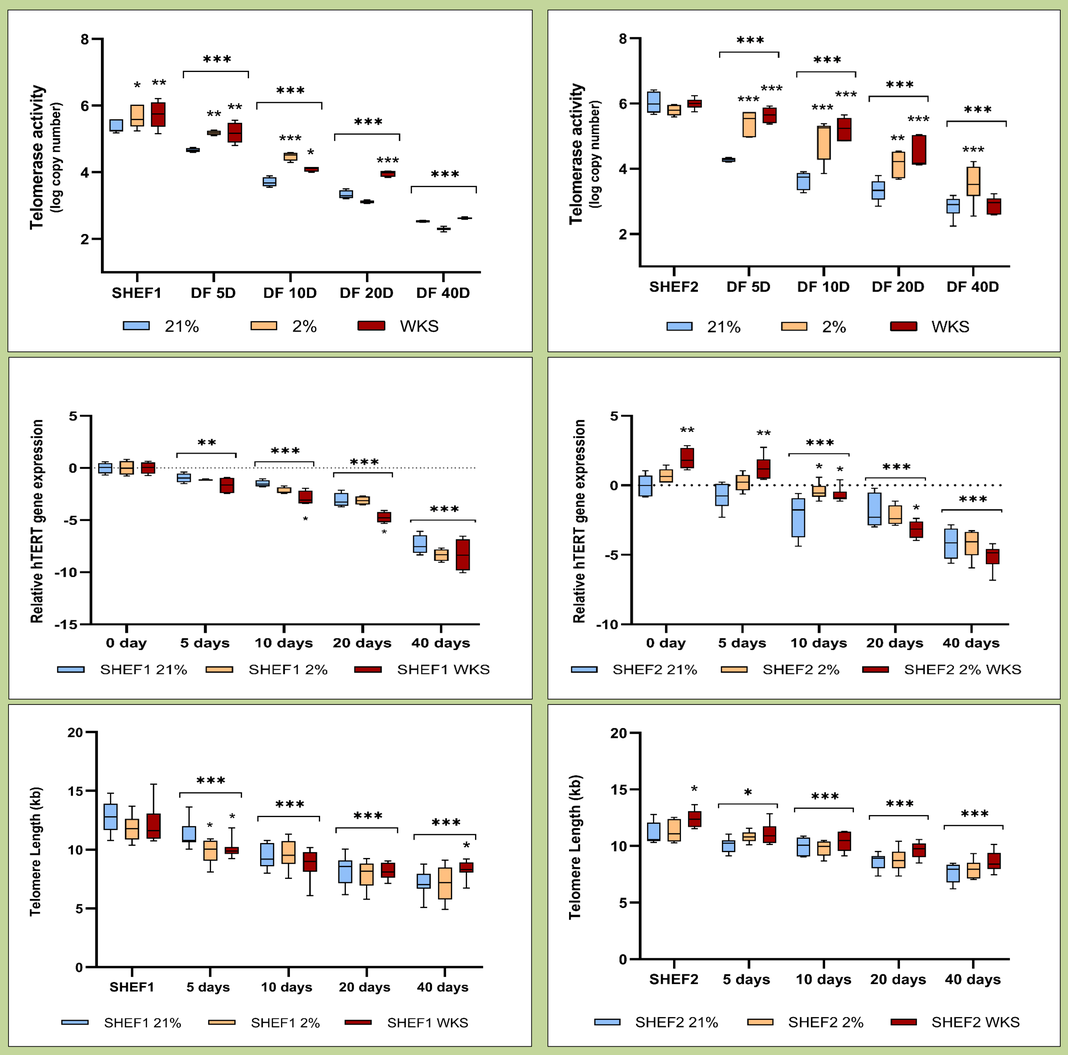


Figure 2. Telomerase activity, TERT gene expression and telomere length detection with Real-time PCR. SHEF1 and SHEF2 cells were obtained from three different conditions at different time points. Data are represented as mean ± standard deviation (SD), n=3, *P<0.05, **P<0.01, ***P<0.001.

Conclusion

In conclusion, promoter hypermethylation seems responsible for downregulated telomerase activity in differentiation process and methylation of hTERT promoter oxygen sensitive. On the other hand, DNMT3B acts as a partial repressor of telomerase enzyme in cancer cells.
A distinct methylation pattern could provide a potential novel therapeutic strategy to inhibit TERT expression without affecting healthy tissue.

References

Lee, D. D., Leão, R., Komosa, M., Gallo, M., Zhang, C. H., Lipman, T., et al. (2019). DNA hypermethylation within TERT promoter upregulates TERT expression in cancer. J. Clin. Invest.
Faleiro, I., Apolónio, J. D., Price, A. J., De Mello, R. A., Roberto, V. P., Tabori, U., et al. (2017). The TERT hypermethylated oncologic region predicts recurrence and survival in pancreatic cancer. Futur. Oncol.

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