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Telomerase and Anticancer Treatment

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Current chemotherapy uses compounds of organometallic nature that act with different mechanisms of action. Many pharmacological studies are directed toward the creation of compounds with more specific and selective activity toward tumor targets, including telomerase. The design and synthesis of such compounds with specific antitelomerase activity must consider the mechanism of action of the enzyme and its structure. The discovery of a close correlation between telomerase activation, cell immortalization and oncogenesis has suggested that telomerase inhibitors could be potent therapeutic agents, capable of selectively killing cancer cells. Inhibition of telomerase is expected to lead toward shortening of telomeres to a critical length, such that replicative senescence and cell death due to irreparable chromosomal damage can result. It has been observed that cancer cells generally have shorter telomeres than the normal replicative cell population, probably because the malignant cells have undergone more divisions. Therefore, the inhibition telomeres of cancer cells after a few cycles of cell division, without the normal cells suffering harmful consequences during therapy. Telomerase is certainly an interesting target on which to continue to study molecules that inhibit its function to obtain a specificity of therapeutic intervention and a reduction of the nonspecific cytotoxicity of chemotherapy.

Keywords: Cancer; hTR; Telomerase; TERT.

In normal human somatic cells, the telomeres, the ends of chromosomes, consist of approximately 15,000 base pairs with a characteristic hexanucleotide repeat sequence, TTAGGG[1,2]. Telomere sequences and their associated proteins form an important structure that prevents chromosome terminal fusion, translocation, and degradation by exonucleases[3-5]. Thus, telomeres preserve the integrity of the chromosome by allowing its complete replication and ensure the proper replication of essential genes[6-8]. Telomeres are progressively shortened at each cell division (loss of about 200 base pairs at each cycle), due to the so-called "terminal replication problem", until they reach a minimum length, called ''crisis'', which produces a strong apoptotic stimulus or the manifestation of a senescent phenotype[9-13]. This phenomenon, termed 'replicative-type senescence'', induces in normal somatic cells the programmed cessation of cell division and limitation of proliferative potential. Therefore, this process has been considered as a mechanism of intrinsic control of tumor suppression: it is hypothesized that it may act as a molecular regulation ("clock") of the amount of cell replication. In cancer cells, telomerase, an RNA-dependent DNA polymerase (retrotranscriptase), adds repetitive DNA sequences

(TTAGGG) to the telomeres, thus preserving the length of chromosomal terminals[14-17]. Thus, telomerase confers unlimited proliferative capacity on the cell, thus playing a central role in the neoplastic process[18-20]. The unlimited proliferative potential that results from the preservation of the telomere length has been defined as one of the six fundamental characteristics that underlie tumor development[21].

Telomerase: Function and Structure

Telomeres are specialised structures of heterochromatin that act as a protective cap for the ends of chromosomes. In most organisms, telomeres consist of repeats of short guanine-rich sequences, complexed with proteins. In humans and other vertebrates, the repeat sequence of telomeres is 5'-TTAGGG-3'.

Each termination of each human chromosome has between 1,000 and 2,000 repeats, with an approximate total of 6-12 kb. The number of repeats and base composition vary between species, but the functions of telomeres are conserved and can be summarized as follows 1) maintenance of chromosomal stability, in terms of protection from the phenomena of degradation by exonucleases, fusion with the ends of other chromosomes by ligases, rearrangement, and recombination; 2) spatial organisation of the cell nucleus and anchoring of the chromosomes themselves during DNA replication; 3) influence on the transcription of genes located in the vicinity of chromosome ends[23,24]. Telomeric DNA has a double-stranded conformation; at the end of the chromosome, however, the guanine-rich 5' 3' strand protrudes 50-150 nucleotides more than the complementary strand rich in cytosine. Telomeric DNA-binding proteins form an intricate multi-protein complex and contribute to maintaining telomere stability and regulating its length. In human cells, the TRF1 and TRF2 proteins specifically recognise the TTAGGG repeat sequence. The function of regulating telomere length, inhibiting its elongation once the critical size has been reached, appears to be assigned to the TRF1 protein. TRF2, on the other hand, hinders the fusion of endings between chromosomes, resulting in remarkable telomere stability. Telomere replication is a late event in the cell cycle and, due to the previously discussed mechanism of DNA replication, incomplete at 5. ends of each newly synthesised strand. At each cell division, human telomeres undergo a loss of approximately 100 base pairs (16 TTAGGG repeats), thus undergoing progressive shortening. After a certain number of mitoses, the telomere reaches a critical length, which triggers a signal to stop cell division and the beginning of a period termed cellular senescence[25].

Telomerase is a ribonucleoprotein belonging to the large family of reverse transcriptases, a polymerase that synthesises DNA from an RNA template; its peculiarity lies in the fact that it has its own RNA template as an integral part of the enzyme. Telomerase binds to the 3' end of eukaryotic chromosomes and adds single-stranded TTAGGG repeat units. Therefore, active telomerase requires two essential components: 1) an RNA subunit, containing a nucleotide sequence that acts directly as a mold for the addition of telomeric repeats; 2) a protein subunit, which is assigned the function of catalyzing telomere synthesis.

The RNA subunit, called TER in general (TR, in mammals; hTR, in humans; TLC1, in yeasts), has a highly conserved secondary structure found in ciliates and vertebrates (Romero and Blackburn, 1991). The human RNA subunit, hTR (human Telomerase RNA) consists of 451 nucleotides, of which eleven represent the CUAACCCUAAC template sequence encoding telomeric repeats (TTAGGG). The coding gene is in the distal part of the long arm of chromosome 3[26-29].

The TERT protein, encoded by the gene of the same name located on chromosome 5 (hTERT in humans, that is, human Telomerase Reverse Transcriptase), is a 127 kDa polypeptide that belongs to the family of reverse transcriptases. Among the characteristic conserved motifs, an important one is a triad of aspartates, which contributes to the formation of binding sites in the polymerase active site; any mutation in one of the aspartates results in altered catalytic functionality. The catalytic subunit of telomerase is conserved in yeast, protozoa and mammals[30].

Furthermore, telomerase-associated proteins appear to play a key role in allowing the enzyme to act in vivo. In yeast, there are at least two proteins associated with the enzyme complex, Est1p and Est3p. However, yeast extracts that have undergone deletion of Est1 or Est3, are capable

of supporting the addition of telomeric repeats in vitro. In the ciliated protozoan Tetrahymena, the p80 protein shows significant homology to a larger protein present in mammalian telomerase, TP1/TEP1. The biological role of these proteins is still being studied. In general, the telomerase complex may consist of many components, each of which is a potential target for the regulation of the enzyme.

Most human somatic cells, except for the hematopoietic system and germ cells, lack telomerase activity and show progressive telomere shortening, resulting in senescence. Telomerase activity is tightly regulated during normal growth and development to avoid unrestricted proliferation directed toward malignancy. Telomerase activity has been observed in several human foetal tissues, such as muscle, lung, and skin, suggesting that the enzyme is active during development and is repressed in adult tissues. The first normal human cells to be telomerase positive were lymphocytes subjected to proliferation stimulation. Other types of normal human cells that exhibit telomerase activity include the intestinal epithelium, the oesophageal epithelium, the endometrium, the basal keratinocytes, the cervix epithelium and stem cells of the hemotopoietic system[31-35].

Recent data show that, within each tissue, a small subpopulation still maintains low levels of telomerase activity, albeit to an extent insufficient to prevent telomere erosion. Studies on the expression of the catalytic subunit of human telomerase (hTERT) in normal tissues agreed with the data obtained from the measurement of telomerase activity by the TRAP assay. Analysis by in situ hybridization revealed the presence of hTERT in a variety of normal cells and tissues with high proliferative capacity, such as different types of epithelial cells, hematopoietic precursors, and spermatogonia. Finally, immunohistochemical studies using anti-hTERT antibodies showed the expression of hTERT in normal colon proliferative epithelial cells[36-39].

In most normal cells, where telomerase activity is lacking, the expression of hTERT cannot even be detected, while hTR and some of its associated proteins are still detectable. These observations suggest that hTERT is an essential factor in telomerase activity and that its regulation plays a key role in the process of cellular immortalisation. The mechanism by which telomerase is repressed in normal somatic cells and is instead activated in tumour cells appears to involve regulation of hTERT expression but is still far from being elucidated. Analysis of hTERT expression has suggested the presence of at least two mechanisms of regulation of telomerase activity: 1) transcriptional control of the hTERT gene; 2) alternative splicing of hTERT transcripts. Recent studies have shown that the hTERT promoter is inactive in normal human somatic cells, while it is activated during immortalisation; detailed analysis of the promoter sequence has revealed binding sites for several transcription factors. The existence of hTERT transcripts, which result in the formation of a truncated or inactive form of the protein, indicates that, in addition to transcriptional activation/repression of the hTERT gene, telomerase activity may be regulated through the alternative splicing mechanism[35,40].

Telomerase and chemotherapy

A new methodological approach based on molecular biology techniques could allow the identification of new and more selective tumor targets. New chemotherapeutic strategies could reduce the cytotoxicity on normal cells and overcome the resistance of tumor cells. Cancerogenesis is a complex phenomenon in which genetic alterations precede the appearance of cytohistological alterations. Therefore, an extensive in vivo evaluation of the relationships between the

Table 1. Features of normal, cancerous, and hTERT-expressing fibroblasts

Characteristics	Normal	Cancer	hTERT
Contact inhibition of growth	present	absent	present
Anchorage-dependence	present	absent	present
Cell cycle checkpoints	present	absent	present
Proliferative life-span	finite	indefinite	indefinite

biomolecular events of the carcinogenesis process and the cytohistological evidence so far considered as the "gold standard" for the diagnosis of neoplasia is desirable[41]. Consistent data indicate that a series of molecular events, leading to the activation of the telomerase enzyme, are the basis for a process of cellular carcinogenesis. Therefore, this enzyme is a tumor marker whose determination can ensure high diagnostic sensitivity and specificity (Table I)[42-46].

Most of the most common cancers, such as breast, prostate, lung, liver, pancreatic, and colon cancer, exhibit telomerase activity, highlighting the key role of this enzyme in tumor pathogenesis[47-49]. Telomerase may already be active at a pre-neoplastic stage or, in other cases, its activity gradually increases in parallel with tumor progression[50]. In many cases, telomerase activity is directly proportional to the aggressiveness of the tumor and its ability to metastasize[51].

The telomerase enzyme is now considered a new marker for tumor cell detection and represents a potential target for selective chemotherapy. Inhibitors of this enzyme, in fact, could cause a progressive reduction of telomeres in tumor cells alone, until a minimum length of the crisis is reached, inducing a strong apoptotic stimulus[52-54].

Understanding the regulatory mechanisms underlying the expression and activity of telomerase could also accelerate the development of new therapeutic modalities for cancer therapy. A telomerase inhibitory strategy would have several advantages, such as directly limiting tumor growth, acting synergistically with existing inhibitors and amplifying their efficiency, it could be used, after initial chemotherapy or surgery, as an adjuvant to block neoplastic cell recovery by increasing susceptibility to immune system activity or killing with traditional chemotherapeutic agents. Moreover, this antitelomerase strategy would be particularly interesting in situations of developing cell turnover, resulting from the use of angiogenesis inhibitors. Systemic administration of telomerase inhibitors could affect the activity present in stem and germ cells. This effect would be minimal because the telomere length of tumor cells, which is relatively shorter, would reach a critical erosion value before irreversible damage is established in other cell types.

Criteria that should be met by a compound with anti-telomerase activity include reducing telomerase activity without initially affecting cell growth capacity; shortening the telomere with each replicative cycle; leading to cell death or tumor growth arrest; initial telomere length influences the time it takes for a reduction in cell proliferation to be observed; reduction in proliferative capacity, or shortening of telomeres should not be due to the action of chemically similar molecules. Many experimental approaches aimed at creating an anti-telomerase strategy have been reported in recent years and the results presented so far seem to encourage research in this direction[55].

Some strategies that were shown to be antitelomerase were not selective, such as: induction of oxidative damage with anticancer drugs such as bleomycin and doxorubicin, directed at telomeres to make them unrecognizable and therefore no longer elongable by telomerase[56-63].

Another nonselective strategy was based on experimental evidence of the formation of paired bases between guanines of the same telomeric chain, which by associating with each other generate cyclic complexes, known as G-quadruplexes[64-65]. Such a G-quadruplex structure could make the telomeric portion inaccessible to telomerase; in fact, the hTERC template RNA region requires an unwounded DNA primer for telomere extension to be possible. Therefore, molecules [1,4-2,6- bis(ù-aminoalkanamido) anthracene-9,10-diene and a cationic porphyrin derivative, tetra (N-methyl-4-pyridyl) porphine (TMPyP4)], stabilizing these structures of G-quadruplex, were produced[66-70]. Selective strategies of inhibition of telomerase have involved the use of base analogues [dideoxyguanine (ddG), 3'-azido-3'deoxythymidine (AZT) and carbovir] that would interfere with the neosynthesis of the telomere at the catalytic site of the enzyme[71-76]. Other selective strategies have involved the use of antisense oligonucleotides of TP1 mRNA and DNA antisense oligonucleotides in vitro[77-80]. Recently, a modified oligonucleotide, sequence-specific peptide nucleic acid (PNA), which hybridizes RNA more efficiently than antisense and is more resistant to proteases, has been used in vitro and in vivo in pancreatic cancer cells[81-84]. Finally, another selective telomerase inhibition approach consisted

of the design, based on the hTERT RNA sequence, of hammerhead ribozymes (Rz), RNA chains endowed with autocatalytic activity, consisting of a conserved portion, called the catalytic site, and two tails that have a sequence complementary to that of the target RNA[85-87]. These molecules bind very specifically to their ribonucleic targets, causing them to be catalyzed at particular sites, usually consisting of the GUC, GUU, or CUC triplets.

CONCLUSIONS

In normal adult tissues, telomerase activity and TERT mRNA expression are greatly reduced. As a result, normal cells in culture are short-lived and undergo growth arrest, called senescence. However, in many cancers, TERT gene expression and telomerase activity are prominent. This has led to proposals for the use of telomerase inhibitors for cancer therapy. Telomerase, since it is therefore active in most malignant tumors and only in a low percentage of normal tissues, could be used as a tumor marker and/or prognostic indicator and provides an excellent target for antitumor therapy.

Evidence of a close correlation between telomerase activation, cell immortalization, and oncogenesis suggests that telomerase inhibitors could be potent therapeutic agents, capable of selectively killing cancer cells. Inhibition of telomerase leads to shortening of telomeres to a critical length, such that it results in replicative senescence and cell death due to irreparable chromosomal damage.

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Authors' contributions

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Conflict of interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

REFERENCES

1. Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR. A highly conserved repetitive DNA sequence,(TTAGGG) n, present at the telomeres of human chromosomes. *Proceedings of the National Academy of Sciences*. 1988;**85**(18):6622-6.
2. Al-Hussaniy HA, Alburghaif AH, Naji MA. Leptin hormone and its effectiveness in reproduction, metabolism, immunity, diabetes, hopes and ambitions. *J Med Life*. 2021;**14**(5):600.-5
3. Tommerup H, Dousmanis A, de Lange TI. Unusual chromatin in human telomeres. *Mol Cell Biol*. 1994;**14**(9):5777-85.
4. Zakian VA. Telomeres: beginning to understand the end. *Science*. 1995;**270**(5242):1601-7.
5. Zakian VA. Structure and function of telomeres. *Annu Rev Genet;* **23**:579-604 (1989).
6. Tantbirojn P, Triratanachat S, Trivijitsilp P, Niruthisard S. Human telomerase reverse transcriptase (hTERT) expression in borderline ovarian tumors: an immunohistochemical study. *Med J Med Ass Thail.* 2009;**92**(3):308.
7. Alkuraishy HM, Al-Gareeb AI, Al-hussaniy HA. Doxorubicin-induced cardiotoxicity: molecular mechanism and protection by conventional drugs and natural products. *Int J Clin Oncol Cancer Res*. 2017;**2**(2):31-44.
8. Blackburn EH. Telomerases. *Annu Rev Biochem*; **61**:113-29 (1992).
9. Aragona MA, Maisano RO, Panetta S, Giudice A, Morelli M, La Torre I, La Torre F. Telomere length maintenance in aging and carcinogenesis. *Intern J Oncology.* 2000;**17**(5):981-90.
10. Fu W, Killen M, Culmsee C, Dhar S, Pandita TK, Mattson MP. The catalytic subunit of telomerase is expressed in developing brain neurons and serves a cell survival-promoting function. *Journal of Molecular Neuroscience*. 2000;**14**(1):3-15.
11. Ferrara P, Marrone G, Emmanuele V, Nicoletti A, Mastrangelo A, Tiberi E,et al. Homotoxicological remedies versus desmopressin versus placebo in the treatment of enuresis: a randomised, double-blind, controlled trial. *Pediatr Nephrol.* 2008; **23**(2):269-274.
12. Falsini B, Iarossi G, Chiaretti A, Ruggiero A, Manni L, Galli-Resta L, et al. NGF eye-drops

topical administration in patients with retinitis pigmentosa, a pilot study. *J Transl Med*. 2016;**14**(1):8-14.
13. Oulton R, Harrington L. Telomeres, telomerase, and cancer: life on the edge of genomic stability. Curr Opin Oncol. 2000;12(1):74-81.
14. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science.* 1994; **266**(5193):2011-5.
15. Karlseder J. Telomere repeat binding factors: keeping the ends in check *Cancer Lett*. 2003; **194**(2): 189-197.
16. Triarico S, Romano A, Attinà G, Capozza MA, Maurizi P, Mastrangelo S, et al. Vincristine-Induced Peripheral Neuropathy (VIPN) in Pediatric Tumors: Mechanisms, Risk Factors, Strategies of Prevention and Treatment. *Int J Mol Sci*. 2021;**22**(8):4112.
17. Falsini B, Ziccardi L, Lazzareschi I, Ruggiero A, Placentino L, Dickmann A, et al. Longitudinal assessment of childhood optic gliomas: relationship between flicker visual evoked potentials and magnetic resonance imaging findings. *J Neurooncol.* 2008; **88**: 87-96
18. Nugent CI, Lundblad V. The telomerase reverse transcriptase: components and regulation. *Genes Dev*. 1998; **12**(8):1073-85.
19. Liu JP. Studies of the molecular mechanisms in the regulation of telomerase activity. *FASEB J.* 1999;**13**(15):2091-104.
20. Masutimi K, Hahn WC. Telomerase and tumorigenesis. *Cancer Lett*. 2003; **194**(2):163-72.
21. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000; **100**:57-70.
22. Blackburn EH. Structure and function of telomeres. *Nature*. 1991; **350**(6319):569-73,
23. Chen JL, Blasco MA, Greider CW. Secondary structure of vertebrate telomerase RNA. *Cell*. 2000;**100**(5):503-14.
24. de Oliveira FM, Jamur VR, Merfort LW, Pozzo AR, Mai S. Three-dimensional nuclear telomere architecture and differential expression of aurora kinase genes in chronic myeloid leukemia to measure cell transformation. *BMC Cancer*. 2022;**22**(1):1024.
25. Colangelo D, Osella D. Telomerase inhibition and cancer: might platinum based drugs have a future as anti-telomerase pharmacological approach? *Curr Med Chem*. 2005;**12**(26):3091-102.
26. Bryan TM, Goodrich KJ, Cech TR. Telomerase RNA bound by protein motifs specific to telomerase reverse transcriptase. *Mol Cell*. 2000;**6**(2):493-9.
27. Al-kuraishy AA, Jalil HJ, Mahdi AS, Al-hussaniy HA. General anesthesia in patient with Brain Injury. *Med Pharm J*. 2022; 27;**1**(1):24-34..
28. Wyles SP, Tchkonia T, Kirkland JL. Targeting Cellular Senescence for Age- Related Diseases: Path to Clinical Translation. *Plast Reconstr Surg*. 2022;**150**:20S-26S.
29. Zhang A, Zheng C, Lindvall C, Hou M, Ekedahl J, Lewensohn R, et al. Frequent amplification of the telomerase reverse transcriptase gene in human tumors. *Cancer Res*. 2000;**60**(22):6230-5.
30. Autexier C, Lue NF. The structure and function of telomerase reverse transcriptase. *Annu Rev Biochem*. 2006;**75**:493-517.
31. Atkinson SP, Hoare SF, Glasspool RM, Keith WN. Lack of telomerase gene expression in alternative lengthening of telomere cells is associated with chromatin remodeling of the hTR and hTERT gene promoters. *Cancer Res*. 2005; **65**(17):7585-90 .
32. Faiola F, Liu X, Lo S, Pan S, Zhang K, Lymar E. Dual regulation of c-Myc by p300 via acetylation-dependent control of Myc protein turnover and coactivation of Myc-induced transcription. *Mol Cell Biol*. 2005; **25**(23):10220-34.
33. Meeser A, Bartenhagen C, Werr L, Hellmann AM, Kahlert Y, Hemstedt N, et al. Reliable assessment of telomere maintenance mechanisms in neuroblastoma. *Cell Biosci*. 2022;**12**(1):160..
34. Ruggiero A, Rizzo D, Trombatore G, Maurizi P, Riccardi R. The ability of mannitol to decrease cisplatin-induced nephrotoxicity in children: real or not?. *Cancer Chemother Pharmacol.* 2016; **77**(1):19-26.
35. Al-hussaniy HA, Altalebi RR, Alburagheef A, Abdul-Amir AG. The Use of PCR for Respiratory Virus Detection on the Diagnosis and Treatment Decision of Respiratory Tract Infections in Iraq. *J Pure Appl Microbiol.* 2022;**16**(1):201-6.
36. Gilson E, Roberge M, Giraldo R, Rhodes D, Gasser SM. Distortion of the DNA double helix by RAP1 at silencers and multiple telomeric binding sites. *J Mol Biol*. 1993;**231**(2):293-310.
37. Ruggiero A, Rizzo D, Catalano M, Coccia P, Triarico S, Attiná G. Acute chemotherapy-induced nausea and vomiting in children with cancer: Still waiting for a common consensus on treatment. *J Int Med Res*. 2018; **46**(6):2149-2156.
38. Giordano P, Lassandro G, Barone A, Cesaro S, Fotzi I, Giona F. Use of Eltrombopag in Children With Chronic Immune Thrombocytopenia (ITP): A Real Life Retrospective Multicenter Experience of the Italian Association of Pediatric Hematology and Oncology (AIEOP). *Front Med.* 2020;7:66.

39. Fetoni AR, Ruggiero A, Lucidi D, De Corso E, Sergi B, Conti G, et al. Audiological Monitoring in Children Treated with Platinum Chemotherapy. *Audiol Neurootol.* 2016; **21**(4):203-211.
40. Muntoni A, Reddel RR. The first molecular details of ALT in human tumor cells. *Hum Mol Genet*; 14 Spec No. 2. 2005:R191-6.
41. Dikmen E, Kara M, Dikmen G, Cakmak H, Dogan P. Detection of telomerase activity in bronchial lavage as an adjunct to cytological diagnosis in lung cancer. *Eur J Cardiothorac Surg.* 2003;**23**:194-200.
42. Dhaene K, Van Marck E, Parwaresch R. Telomeres, telomerase and cancer: an up-date. *Virchows Arch*. 2000;**437**(1):1-16.
43. Hiyama E, Hiyama K. Clinical utility of telomerase in cancer. *Oncogene.* 2002;**21**:643-649.
44. Romano A, Capozza MA, Mastrangelo S, Maurizi P, Triarico S, Rolesi R, et al. Assessment and Management of Platinum-Related Ototoxicity in Children Treated for Cancer. *Cancers (Basel)*. 2020;**12**(5):1266.
45. Gao J, Pickett HA. Targeting telomeres: advances in telomere maintenance mechanism-specific cancer therapies. *Nat Rev Cancer;* (2022 Jul 5). doi:10.1038/s41568-022-00490-1.
46. Hiyama E, Hiyama K, Yokoyama T, Shay JW. Immunohistochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. *Neoplasia.* 2001; **3**: 17-26.
47. Meeker AK, Coffey DS. Telomerase: a promising marker of biological immortality of germ, stem, and cancer cells. A review. *Biochemistry*. 1997;**62**(11):1323-31.
48. Ruggiero A, Cefalo MG, Coccia P, Mastrangelo S, Maurizi P, Riccardi R. The role of diet on the clinical pharmacology of oral antineoplastic agents. *Eur J Clin Pharmacol*, 2012;**68**(2):115-122.
49. ALZobaidy MA, AlbuRghaif AH, Alhasany HA, Naji MA. Angiotensin-Converting Enzyme Inhibitors May Increase Risk of Severe COVID-19 Infection. *Annals of the Romanian Society for Cell Biology*. 2021;**25**(6):17843-9.
50. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer.* 1997; **33**:787-791.
51. Avilion AA, Piatyszek MA, Gupta J, Shay JW, Bacchetti S, Greider CW. Human telomerase RNA and telomerase activity in immortal cell lines and tumor tissues. *Cancer Res*. 1996;**56**(3):645-50.
52. Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A. Inhibition of telomerase limits the growth of human cancer cells. *Nat Med*. 1999;**5**(10):1164-70.
53. Trisciuzzi MT, Riccardi R, Piccardi M, Iarossi G, Buzzonetti L, Dickmann A, et al. A fast visual evoked potential method for functional assessment and follow-up of childhood optic gliomas. *Clin Neurophysiol.* 2004;**115**: 217-226.
54. Saretzki G. Telomerase inhibition as cancer therapy. *Cancer Lett*. 2003; **194**(2):209-19.
55. Pendino F, Tarkanyi I, Dudognon C, Hillion J, Lanotte M, Aradi J, et al. Telomeres and telomerase: Pharmacological targets for new anticancer strategies? *Curr Cancer Drug Targets*. 2006; **6**(2):147-80.
56. Aldossary SA. Review on pharmacology of cisplatin: clinical use, toxicity and mechanism of resistance of cisplatin. *Biomed Pharmacol J*. 2019;**12**(1):7-15.
57. Sabbah MF, Alshubali F, Baothman OA, Zamzami MA, Shash L, Hassan IA. Cardioprotective Effect of Ajwa Date Aqueous Extract on Doxorubicin-Induced Toxicity in Rats. *Biomed Pharmacol J*. 2018;**11**(3):1521-36.
58. Posteraro B, Bruno S, Boccia S, Ruggiero A, Sanguinetti M, Romano Spica V, et al. Candida parapsilosis bloodstream infection in pediatric oncology patients: results of an epidemiologic investigation. *Infect Control Infect Control Hosp Epidemiol*. 2004;**25**(8):641-5.
59. Pakhomova T, Moshareva M, Vasilkova D, Zatsepin T, Dontsova O, Rubtsova M. Role of RNA Biogenesis Factors in the Processing and Transport of Human Telomerase RNA. *Biomedicines*. 2022;**10**(6):1275.
60. Prathap L, Kumar P, Preetha S. Effect of Physical Exercise in Remodeling Telomere Length and Cancer Prevention in an Epigenetic Prospect–A Systematic review. *Biomed Pharmacol J*. 2021;**14**(2):891-901.
61. Revy P, Kannengiesser C, Bertuch AA. Genetics of human telomere biology disorders. *Nat Rev Genet*. 2022. doi: 10.1038/s41576-022-00527-z.
62. Lazzareschi I, Ruggiero A, Riccardi R, Attinà G, Colosimo C, Lasorella A. Hypersensitivity reactions to carboplatin in children. *J Neurooncol.* 2002; **58**:33-37.
63. Von Zglinicki T, Saretzki G, Docke W, Lotze C. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res.* 1995;**220**(1):186-93.
64. Wright WE, Tesmer VM, Huffman KE, Levene SD, Shay JW. Normal human chromosomes have long G-rich telomeric overhangs at one end. *Genes Dev*. 1997;**11**(21):2801-9.
65. Wellinger RJ, Sen D. The DNA structures at the ends of eukaryotic chromosomes. *Eur J Cancer.*

1997; (5):735-49.
66. Salazar M, Thompson BD, Kerwin SM, Hurley LH. Thermally induced DNA. RNA hybrid to G-quadruplex transitions: possible implications for telomere synthesis by telomerase. *Biochemistry*. 1996;17;**35**(50):16110-5.
67. Shi DF, Wheelhouse RT, Sun D, Hurley LH. Quadruplex-interactive agents as telomerase inhibitors: synthesis of porphyrins and structure-activity relationship for the inhibition of telomerase. *J Med Chem.* 2001; **44**(26):4509-23.
68. Jenner LP, Peska V, Fulneèková J, Sýkorová E. Telomeres and Their Neighbors. *Genes (Basel).* 2022;**13**(9):1663.
69. Sun D, Thompson B, Lathers BE, Salazar M. Inhibition of human telomerase by a G-quadruplex interactive compound. *J Med Chem.* 1997; **40**:2113-6.
70. Zahler AM, Williamson JR, Cech TR, Prescott DM. Inhibition of telomerase by G-quartet DNA structures. *Nature*. 1991;**350**(6320):718-20.
71. Strahl C, Blackburn EH. Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortalized human cell lines. *Mol Cell Biol*. 1996;**16**(1):53-65.
72. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell*. 1997;**88**(3):323-31.
73. Chiaretti A, Ruggiero A, Barbi E, Pierri F, Maurizi P, Fantacci C, et al. Comparison of propofol versus propofol-ketamine combination in pediatric oncologic procedures performed by non-anesthesiologists. *Pediatr Blood Cancer.* 2011;**57**(7):1163-1167.
74. Yegorov YE, Chernov DN, Akimov SS, Bolsheva NL, Krayevsky AA, Zelenin AV. Reverse transcriptase inhibitors suppress telomerase function and induce senescence-like processes in cultured mouse fibroblasts. *FEBS Lett*. 1996; **389**(2):115-8.
75. Melana SM, Holland JF, Pogo BG. Inhibition of cell growth and telomerase activity of breast cancer cells in vitro by3'-azido-3'- deoxythymidine. *Clin Cancer Res*. 1998;**4**(3):693-6.
76. Collins K, Gandhi L. The reverse transcriptase component of the Tetrahymena telomerase ribonucleoprotein complex. *Proc Natl Acad Sci.* 1998; **95**(15): 8485-90.
77. Strahl C, Blackburn EH. The effects of nucleoside analogs on telomerase and telomeres in Tetrahymena. *Nucleic Acids Res.* 1994;**22**(6):893-900.
78. Cefalo MG, Maurizi P, Arlotta A, Scalzone M, Attinà G, Ruggiero A, et al. Hepatic veno-occlusive disease: a chemotherapy-related toxicity in children with malignancies. *Paediatr Drugs.* 2010;**12**(5):277-84.
79. Rizzo D, Scalzone M, Ruggiero A, Maurizi P, Attinà G, Mastrangelo S, et al. Temozolomide in the treatment of newly diagnosed diffuse brainstem glioma in children: a broken promise?. *J Chemother.* 2015;**27**(2):106-110.
80. Sharma S, Chowdhury S. Emerging mechanisms of telomerase reactivation in cancer. *Trends Cancer.* 2022;S2405-8033(22)00071-1.
81. Glukhov AI, Zimnik OV, Gordeev SA, Severin SE. Inhibition of telomerase activity of melanoma cells in vitro by antisense oligonucleotides. *Biochem Biophys Res Commun*. 1998; **248**(2):368-71.
82. Norton JC, Piatyszek MA, Wright WE, Shay JW, Corey DR. Inhibition of human telomerase activity by peptide nucleic acids. *Nat Biotechnol*. 1996;**14**(5):615-9.
83. Ruggiero A, Barone G, Liotti L, Chiaretti A, Lazzareschi I, Riccardi R. Safety and efficacy of fentanyl administered by patient-controlled analgesia in children with cancer pain. *Support Care Cancer*. 2007;**15**(5):569-73.
84. Naka K, Yokozaki H, Yasui W, Tahara H, Tahara E, Tahara E. Effect of antisense human telomerase RNA transfection on the growth of human gastric cancer cell lines. *BiochemBiophys Res Commun.* 1999; **255**(3):753-8.
85. Yokoyama Y, Takahashi Y, Shinohara A, Lian Z, Wan X, Niwa K, et al. Attenuation of telomerase activity by a hammerhead ribozyme targeting the template region of telomerase RNA in endometrial carcinoma cells. *Cancer Res.* 1998;**58**(23):5406-10.
86. Brenner KA, Nandakumar J. Consequences of telomere replication failure: the other end-replication problem. *Trends Biochem Sci*. 2022;**47**(6):506-517.
87. Quazi S. Telomerase gene therapy: a remission toward cancer. *Med Oncol*. 2022;**39**(6):105.