

REVIEW

Effect of chemotherapeutic drugs on telomere length and telomerase activity

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Telomeres are specialized nucleoproteic complexes localized at the ends of eukaryotic chromosomes, that maintain their stability and integrity. They protect chromosome ends from fusion and from being recognized as sites of DNA damage, i.e., they distinguish natural DNA ends from DNA ends resulting from breakage events. In mammalian cells, telomeres consist of tandem arrays of the hexanucleotide TTAGGG, oriented 5' to 3' towards the end of the chromosomes and associated proteins (the so-called “shelterin” complex), and a large non-coding RNA (named TERRA) which forms an integral component of telomeric heterochromatin. Telomere length is maintained by a dynamic process of telomere shortening and lengthening. Shortening can occur due to nucleolytic degradation and incomplete DNA replication due to the inability of lagging strand synthesis to completely replicate chromosomal ends (i.e., the “end replication problem”), whereas lengthening is primarily accomplished by the action of the enzyme telomerase and occasionally by the so-called Alternative Lengthening of Telomeres (“ALT”) mechanism, which involves homologous recombination. The maintenance of telomere function is crucial for genomic stability and cell viability. Cells respond to dysfunctional telomeres by undergoing senescence, cell death, or genomic instability. Since telomeres play a fundamental role in maintaining chromosomal/genomic stability and telomerase activity and telomere lengthening play a key role in cancer development and progression, a proper knowledge of the effects of chemotherapeutic drugs on telomere length and telomerase activity in normal as well as tumor cells is of great importance to understand the genomic instability associated with chemotherapy regimens. Therefore, in this review we will summarize our current knowledge concerning the main data available about the effects of chemotherapeutic drugs on telomere length and telomerase activity in mammalian cells.

Keywords: Telomere; telomere length; telomerase; anticancer drugs; chemotherapeutic drugs

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Introduction

Telomeres are specialized nucleoproteic complexes localized at the ends of eukaryotic chromosomes, that

maintain their stability and integrity^[1-3]. They protect chromosome ends from fusion and from being recognized as sites of DNA damage, i.e., they distinguish natural DNA from DNA ends resulting from breakage events^[1-3].

In all vertebrates, telomeres are composed of tandem arrays of the hexanucleotide TTAGGG, oriented 5' → 3' towards the end of the chromosome, ending in a 3' single-stranded overhang ranging in length from ~50 to 400 nt^[4-6], bound by a specialized multiprotein complex known as “shelterin”^[7], constituted by 6 proteins (POT1, TPPI. TIN2, TRF1, TRF2 and RAP1). Shelterin proteins are charged with protecting chromosome ends from activating a DNA damage response, inhibiting inappropriate repair mechanisms, and maintaining telomeric length and structure^[7]. Telomeres also comprise (UUAGGG)_n-containing RNA molecules (TERRA), a long non-coding RNA that is transcribed from telomeric DNA by RNA polymerase II^[8-9]. TERRA has been implicated in several processes related to telomere function, including telomerase regulation, telomere chromatin organization and gene expression^[8-10].

Telomere length is maintained by a dynamic process of telomere shortening and lengthening. The telomeres are progressively lost with each round of cell division, losing approximately 20-300 bp of repeat sequences every cell division mainly due to the “end replication problem” (i.e., the inability of DNA polymerase to replicate DNA to the ends of linear chromosomes)^[3]. This is the main mechanism for the loss of telomeric repeats and is termed replicative erosion or replicative shortening, which leads to replicative senescence of cells. Telomere erosion refers to a dysfunctional telomere (i.e., a telomere which has lost their end-capping function) which became critically short, so it cannot function properly. There is another kind of telomere shortening, termed “stress-dependent shortening”, which is produced by stress-inducing factors like radiation, oncogenes, oxidative damage within telomeric DNA, and chromosome end-specific exonuclease activity^[11-14]. Telomere shortening can promote genome instability, which may lead to cancer^[3, 15].

To avoid telomere shortening, some cells activate telomerase, a reverse transcriptase (i.e., a RNA dependent polymerase) that elongates chromosomes by adding TTAGGG sequences to the end of chromosomes. Telomerase is a ribonucleoprotein composed of dyskerin, the catalytic subunit termed TERT, and the RNA subunit named TERC, which serve as template to synthesize telomere DNA^[3, 10, 16]. Although inactive in most somatic cells -with the exception of a transient S phase activity thought to maintain the single-stranded overhang- telomerase is active in immortal cell lines, germline cells, stem cells, activated lymphocytes, and most of the tumor cells analyzed so far^[10, 16]. Loss of telomerase enzymatic function leads to progressive telomere shortening over time, eventually resulting in the disappearance of detectable telomeric DNA and the formation of end-to-end chromosome fusions, followed by growth arrest

or cell death^[10, 16, 17]. In addition to telomere length maintenance, telomerase is also involved in other cellular processes not directly related to telomere function, including gene expression regulation, cell proliferation, apoptosis and cell signaling, adhesion and migration^[10]. All these activities of telomerase are thought to contribute significantly to the process of carcinogenesis. In fact, telomerase upregulation or reactivation is a critical feature in over 90 % of cancers^[10]. Because of this, telomerase has been a prime target for the development of effective therapeutics against cancer^[10].

Telomere elongation can also occur in the absence of telomerase through the so-called ALT (for ‘alternative lengthening of telomeres’) mechanism, which involves homologous recombination between telomeres and has been described in several tumor cells and immortalized cell lines^[17, 18]. The ALT mechanism is employed by 10-15 % of tumors^[17, 18].

Many drugs used in conventional chemotherapy for the treatment of cancer induce genome instability and affect telomere function. Thus, several studies have shown that cancer patients treated with chemotherapeutic agents and radiation have shorter telomere in their blood cells, even after the end of the treatments^[19-24]. Because telomere plays a critical role in regulating cellular life-span, aging and genomic stability, telomere shortening or erosion likely contributes to late side-effects occurring in long-term survivors of cancer patients. It has been suggested that repeated exposure to chemotherapy and radiotherapy may cause telomere dysfunction through which organ impairment and genomic instability take place^[25]. Thus, telomere dysfunction will eventually contribute to undesirable side-effects in long-term survivors of cancer patients, such as the development of secondary tumors. Since telomeres play a fundamental role in maintaining chromosomal/genomic stability and telomerase activity and telomere lengthening play a key role in cancer development and progression^[10], a proper knowledge of the effects of chemotherapeutic drugs on telomere length and telomerase activity in normal as well as tumor cells is of great importance to understand the genomic instability associated with chemotherapy regimens. Therefore, in this review, we will summarize our current knowledge concerning the main data available about the effects of chemotherapeutic drugs on telomere length and telomerase activity in mammalian cells.

Effect of chemotherapeutic drugs on telomere length and telomerase activity

In this section, we will consider the main data available concerning the short- and long-term effects of chemotherapeutic drugs on telomere length and telomerase

Table 1. Effects of chemotherapeutic agents on telomere length and telomerase activity in mammalian cells

Drug	Telomere length	Telomerase activity	Cell type and references
Bleomycin	ND	--	Chinese hamster cells [32]
	↓↑-- (*)	--↑↓ (*)	Rat cells (fibroblasts derived from dedifferentiated adipose cells) [34, and unpublished data]
	ND	↑	Rat cells (lung fibroblasts) [38]
	ND	↑	Mouse cells (lung epithelial cells) [37]
	ND	--	Human testicular cancer cells [39]
Streptonigrin	--	--	Mouse spermatogonial cells [40]
	ND	--	Chinese hamster cells [32]
	↓↑-- (*)	↓	Rat cells (fibroblasts derived from dedifferentiated adipose cells) [35, and unpublished data]
Streptozotocin	ND	--	Chinese hamster cells [33]
	--↑-- (*)	--	Rat cells (fibroblasts derived from dedifferentiated adipose cells) [36]
Paclitaxel	↓	--	Mouse melanoma cells [42]
	↓	--	Paclitaxel-requiring mutant CHO cells [43]
	↓	↓	Human pharynx tumor cells [45]
Paclitaxel + AZT	--	--	Human ovarian cancer cells [44]
	↓	ND	Human pharynx tumor cells [46]
Cisplatin	↓	ND	Hela cells [51]
	ND	↓	Human testicular cancer cells [39]
	ND	↓	Human endometrial cancer cells [48]
	↓	↓	Mouse spermatogonial cells [40]
	↓	↑	Human ovarian cancer cells [44]
	↓	↓	Human hepatoma cells [49]
	ND	↓	Human ovarian cancer cells [50]
	ND	↓	Human head and neck squamous cell carcinoma cell lines [52]
Doxorubicin	ND	--	Human haematopoietic cancer cell lines [53]
	↓	↓	Human hepatoma cells [49]
	--	--	Human ovarian cancer cells [44]
	--	↓	Human breast tumor cells [54]
	ND	↓	Human breast and stomach cancer cells [55]
	↓	↓	Human normal T-lymphocytes and fibroblasts [25]
	ND	↓	Human ovarian cancer cells [50]
Etoposide	ND	--	Human testicular cancer cells [39]
	--	--	Mouse spermatogonial cells [40]
	↓	↑	Human ovarian cancer cells [44]
	--	ND	Human neuroblastoma and acute lymphoblastic T cells [58]
	ND	--	Human haematopoietic cancer cell lines [53]
	↓	↓	Human normal T-lymphocytes and fibroblasts [25]
	ND	↑	Human leukemic cells [59,60]
	ND	↑	Human pancreatic tumor cell lines [61]
	ND	↓	Human hepatomas and leukemic cell lines [62]
	ND	--	Human nasopharyngeal carcinoma cells [63]
Etoposide + Doxorubicin	↓	ND	Human mesenchymal stem cells [56]
Etoposide + bleomycin + cisplatin	↓	ND	Rat male germ cells [57]
Azydothimidine (AZT)	↓	↓	Human lymphoid cell lines [65]
	↓	ND	Hela cells [67]
	↓	↓	Human endometrial carcinoma cells [66]
	ND	↓	Mouse mammary carcinoma cells [68]
	ND	↓	Human pharynx cells [45]
	ND	↓	Human parathyroid cancer cells [69]
	ND	↓	Human liver carcinoma cells [70]
	ND	↓	Mouse mammary carcinoma cells [64]
AZT + arsenic trioxide	ND	↓	Human acute promyelocytic leukemia cells [72]
AZT + 5-FU	ND	↓	Human colon cancer cells [71]
5-Azacytidine	↓	↓ (expression of TERT)	Acute myeloid leukemia cells [75]
	--	ND	Human glioblastoma cells [76]
5-Aza-2'deoxyctidine ± Trichostatin A	↑	ND	Breast cancer cells [74]
Gemcitabine	↓	--	Hela cells [78]
	↓	ND	Human colon cancer cells [83]
	↓	ND	Human osteosarcoma cells [85]
	--	ND	Human colon carcinoma and cervical cancer cells [85]

Melphalan	ND ↑ / ↓ (***)	-- ND	Human testicular cancer cells ^[39] Mouse bone marrow cells ^[87]
5-Fluorouracil (5-FU)	ND	--	Human head and neck squamous cell carcinoma cell lines ^[52]
5-FU + Cisplatin	↑	↑	Human colorectal carcinoma cells ^[89]
Cyclophosphamide	↓	↑	Human ovarian cancer cells ^[44]
4OOH-CPA (**)	↓	↓	Mouse spermatogonial cells ^[40]
Mitomycin C	ND	--	Human haematopoietic cancer cell lines ^[53]
Methotrexate	ND	--	Human testicular cancer cells ^[39]

↑: increasing effect (telomere elongation); ↓: decreasing effect (telomere shortening or erosion); --: no effect; ND: Not determined. (*) These symbols represent the effects of the drug observed at 18 h, 10 days and 15 days after treatment, respectively. (**) 4-hydroperoxycyclophosphamide, a preactivated analog of cyclophosphamide. (***) The increasing effect was observed in p53[±] mice, whereas the decreasing effect was observed in wild-type mice.

activity in mammalian cells. First, we will refer to those anticancer drugs whose effects on telomere length and telomerase activity have been intensively investigated and then we will focus on some anticancer drugs whose effects on these parameters are barely known. For a summary of the effects of each drug on telomere length and telomerase activity, the reader should refer to Table 1.

Bleomycin, streptonigrin and streptozotocin

Several years ago, we carried out a series of experiments to determine the effects of three antitumoral antibiotics, namely, bleomycin, streptonigrin and streptozotocin, on mammalian telomeres and telomeric sequences (see^[26] for review).

Bleomycin (CAS No. 11056-06-7) is a chemotherapeutic drug isolated from *Streptomyces verticillus* which is commonly used to treat testicular cancer, non-Hodgkin lymphoma, lung cancer, cervical cancer and cancers of the head and neck^[27]. This antibiotic is a radiomimetic agent that generates free radicals and induces single- and double-strand breaks in DNA^[27, 28]. Streptonigrin (CAS No. 3930-19-6) is an aminoquinone antitumor antibiotic isolated from cultures of *Streptomyces flocculus*, which shows antitumor activity against a broad range of tumors, including breast, lung, head and neck cancer, lymphoma and melanoma^[29], although its use in chemotherapy is very limited because of the severe and prolonged bone marrow depression it induces^[29]. Streptonigrin is also a venom of topoisomerase II, inhibiting this enzyme by stabilizing its transesterification intermediate (cleavable complex)^[29]. Streptozotocin or streptozocin (CAS No. 18883-66-4) is an antibiotic isolated from *Streptomyces achromogenes*^[30, 31], usually used to experimentally induce diabetes mellitus in rats, and considered a potential compound for the clinical treatment of some malignant diseases, specially advanced pancreatic neuroendocrine tumors and colon cancer. Also, streptozotocin is a potent alkylating agent that directly methylates DNA^[30, 31].

Despite the abovementioned compounds induced chromosomal aberrations involving telomeres and interstitial

telomeric sequences, none of them altered the activity of telomerase in CHO and CHE cells^[32, 33], at least in the short-term (18 h after treatment). The effect of these drugs on telomere length in hamster cells was not determined. More recently, we investigated the long-term effect (i.e., up to 15 days after treatment) of bleomycin, streptonigrin and streptozotocin on telomeres of rat cells (the ADIPO-P2 cell line, derived from dedifferentiated adipose cells from Sprague-Dawley rats)^[34-36]. Bleomycin and streptonigrin had a variable effect on telomere length, since they both induced telomere shortening at 18 h, telomere elongation at 10 days, and had no effect on telomere length at 15 days after treatment (Paviolo, unpublished). Nevertheless, these compounds had a completely different effect on telomerase activity. Bleomycin did not modify the activity of telomerase at 18 h after treatment (the same effect previously observed in Chinese hamster cells^[32]), but produced an increase at 10 days and a decrease at 15 days after treatment^[34]. Thus, bleomycin produced a delayed induction of telomerase activity in mammalian cells. This finding is in line with that of Fridlender *et al.*^[37] who found that this compound induces telomerase activity in lung epithelial cells of mice 7 and 14 days after treatment, and that of Nozaki *et al.*^[38] who found elevated levels of telomerase in fibroblasts and tissue extracts isolated from bleomycin-injured rat lungs at 7, 14, and 21 days posttreatment, with maximal activity observed in the day 14 samples. Therefore, we concluded that the observed telomere instability induced by bleomycin in rat cells was unrelated to telomerase activity^[34].

On the contrary, streptonigrin induced a persistent inhibition of telomerase activity (up to 77%) in streptonigrin-treated rat cells in comparison with that of untreated (control) rat cells at each time points analyzed (18 h, 10 days and 15 days after treatment). Therefore, telomerase could be involved in the observed telomere dysfunction in these cells (through the induction of telomere-related chromosomal aberrations^[35]), but this assumption requires further studies to be confirmed. We previously observed in Chinese hamster cells that telomerase activity in streptonigrin-treated cells was similar to that of untreated cells 18 h after treatment^[32]. Thus, the effect of

streptonigrin on telomerase activity seems to be dependent on the cell type.

Concerning streptozotocin, we found that it also has a variable effect on telomere length in the long-term, since this drug had no significant effect at 18 h and 15 days after treatment, but induced telomere elongation at 10 days after treatment. These effects were not related with telomerase activity, which remained unchanged in both treated and untreated cells from 18 h to 15 days after treatment [36]. Therefore, the persistence of chromosomal aberrations related to telomere dysfunction observed in rat cells exposed to streptozotocin seems to be unrelated to telomerase activity or telomere length. The data registered in telomerase activity in the short-term agrees well with the data previously obtained in CHO cells, where no variations of the activity were observed 18 h posttreatment with streptozotocin [33] in comparison to control (unexposed) cells.

Besides the abovementioned studies, other researchers reported additional data on the effect of bleomycin on telomere length and telomerase activity. Burger *et al.* showed that bleomycin inhibits telomerase activity in human testicular cancer cells [39]. Recently, Liu *et al.*, studied the effect of bleomycin and other chemotherapeutic drugs on telomeres of a mouse spermatogonial cell line and found that this compound did not affect neither telomere length nor telomerase activity in these cells [40]. However, bleomycin induced telomere DNA damage in these cells, as seen by the colocalization of the telomere FISH probe signal with immunofluorescent γ H2AX foci (a marker of DNA double-strand breaks) [40]. No further studies have been made concerning the effects of streptonigrin or streptozotocin on telomeres so far.

Paclitaxel

Paclitaxel or Taxol (CAS No. 33069-62-4) is an anticancer drug, isolated from the bark of the Pacific yew *Taxus brevifolia*, that has been shown to be clinically effective against ovarian, breast, lung, pancreatic, and other human cancers [41].

It has been shown that paclitaxel and its water-soluble forms poly (L-glutamic acid)-paclitaxel, sodium-pentetic acid-paclitaxel, and polyethylene glycol-paclitaxel, induced extensive telomere erosion (visualized as reduced telomeric signal intensity after telomere fluorescent *in situ* hybridization) in a murine metastatic melanoma cell line (K1735, clone X-21), but did not affect telomerase activity [42]. Similar results were also observed in Tax-18 and Tax-2-4, two paclitaxel-requiring mutant CHO cell lines [43]. Moreover, paclitaxel did not affect telomere length or

telomerase activity in human ovarian cancer cells [44]. Interestingly, telomere erosion induced by paclitaxel can be enhanced by telomerase inhibitors, such as AZT, as demonstrated in human pharynx FaDu tumor cells [45, 46].

Cisplatin

Cisplatin (CAS No. 15663-27-1) is a well-known platinum-based anticancer drug with alkylating properties, which forms DNA adducts and induces apoptosis [47]. This compound is used alone or in combination with other chemotherapeutic agents for the clinical treatment of several types of cancers, including testicular, ovarian and lung cancer, among others [47].

The inhibitory effect of cisplatin on telomerase activity was demonstrated in several types of cells, including human testicular cancer cells [39], endometrial cancer cells [48], hepatoma cells [49], ovarian cancer cells [50] and mouse spermatogonial cells [40]. In the study from Liu *et al.* [40], it was also found that cisplatin shortens telomere length, thus causing telomere dysfunction. Thus, cisplatin could induce long-term telomeric loss in mammalian cells, resulting from the inhibition of the enzyme telomerase. Moreover, Ishibashi and Lippard [51] by using Analysis of TRF Length (by Southern blot) showed that cisplatin induces telomere loss (shortening) and degradation in HeLa cells. Cisplatin was also found to induce telomere shortening in human hepatoma cells [49] and human ovarian cancer cells [44]. However, the effect of cisplatin on telomerase activity in human ovarian cancer cells is not clear, since one study showed that this compound increases telomerase activity [44], whereas another study showed that cisplatin inhibited telomerase activity in these cells [50]. Moreover, cisplatin was found to inhibit telomerase activity in human neck and head squamous cell carcinoma cell lines [52]. No effect of cisplatin on telomerase activity in human haematopoietic cancer cell lines was observed [53].

Doxorubicin and etoposide

Doxorubicin (also called Adriamycin, CAS No. 23214-92-8) and etoposide (CAS No. 33419-42-0) are both topoisomerase II inhibitors with anticancer properties. Doxorubicin inhibits telomerase activity in human testicular cancer cells [39]. It has been found that doxorubicin inhibits telomerase activity and shortens mean telomere length in human hepatoma cells [49]. Thus, it has been proposed that telomerase inhibition and telomere shortening by doxorubicin may contribute to its efficiency in the treatment of hepatocellular carcinoma. However, doxorubicin had no effect on telomerase or telomere length in human ovarian cancer cells [44] or inhibited telomerase activity in these cells

(cell line A2780)^[50], decreased telomerase activity but had no effect on telomere length in breast tumor cells^[54], and decreased telomerase activity in several human breast and stomach cancer cell lines^[55]. Thus, the effect of doxorubicin on telomeres depends on the cell type.

A few years ago, it was reported that doxorubicin and etoposide induce progressive telomere shortening in human mesenchymal stem cells, obtained from bone marrow cells from normal adults and grown in the presence of platelet lysates^[56]. A year later, Li *et al.* reported that the treatment of normal human T lymphocytes and fibroblasts with doxorubicin or etoposide led to significant shortening of telomeres, down-regulation of telomerase activity, diminished expression of TERT and the telomere binding proteins TPP1 and POT1 and telomere dysfunction in these cells^[25]. However, recent data reported by Liu *et al.* showed that etoposide alone does not specifically affects telomeres of a mouse spermatogonial cell line and that this drug did not induce telomere dysfunction in these cells^[40], but in combination with bleomycin and cisplatin, etoposide produces telomere shortening in rat male germ cells^[57]. In addition, it was demonstrated that etoposide did not affect telomere length in the neuroblastoma cell line SHSY5Y (with very short telomeres) or the acute lymphoblastic T cell line 1301 (which exhibits extremely long telomeres)^[58]. Moreover, etoposide has no effect on telomerase activity in human haematopoietic cancer cell lines^[53] and induces telomere shortening and decreases telomerase activity in human ovarian cancer cells^[44]. Upregulation of telomerase activity was observed in the human leukemic cell line HL60^[59,60], and several pancreatic tumor cell lines after etoposide treatment^[61]. However, a decrease in telomerase activity was observed in hepatocarcinomas and other leukemic cell lines^[62] and no change in levels of telomerase in nasopharyngeal carcinoma cells^[63]. Thus, as with doxorubicin, the effect of etoposide on telomeres depends on the cell type.

AZT

Azidothymidine or zidovudine (CAS No. 9003-98-9), is a thymidine analog used in the treatment of AIDS, that also exhibits anticancer effects^[64]. It has been found that AZT inhibits telomerase activity and causes telomere shortening in human lymphoid cell lines (the B cell line JY616 and the T cell line Jurkat E6-1)^[65] and human endometrial carcinoma cells^[66], produces irreversible telomere shortening in HeLa cells^[67], and inhibits telomerase activity in mouse mammary carcinoma cells^[68], human pharynx cells^[45], human parathyroid cancer cells^[69], human liver carcinoma cells^[70] and mouse mammary carcinoma cells^[64]. In addition, AZT in combination with arsenic trioxide or 5-FU also inhibits telomerase activity in human colon cancer cells^[71] and acute

promyelocytic leukemia cells^[72]. Thus, AZT clearly inhibits telomerase activity in mammalian cells.

5-AZA

5-azacytidine (5-AZA, Ladakamycin, CAS No. 320-67-2) and its deoxy derivative 5-aza-2'-deoxycytidine (Decitabine, CAS No. 2353-33-5) are demethylating compounds (inhibit DNA methyltransferases) with anticancer properties, usually employed against myelodysplastic syndrome and acute myeloid leukemia^[73]. It has been shown that 5-aza-2'-deoxycytidine, either alone or in combination with trichostatin A, induces telomere elongation in breast cancer cell lines due to up-regulation of shelterin genes^[74]. In addition, 5-AZA was found to induce DNA damage at telomeres, telomere shortening, diminished TERT expression and apoptosis in acute myeloid leukemia cell lines^[75]. Thus, it was suggested that another mechanism (besides DNA demethylation) by which 5-AZA exerts its antitumoral activity is telomere dysfunction^[75]. On the contrary, Choudhury *et al.*, using the glioblastoma cell line SF-767, found that 5-AZA caused significant changes in DNA methylation of subtelomeric regions of chromosomes but did not modify the telomere length in these cells^[76]. Thus, further studies will be needed to clarify the effect of this compound on telomere length and telomerase activity.

Gemcitabine

It has been recently reported that the cytidine analog gemcitabine (2',2'-difluorodeoxycytidine) (CAS No. 95058-81-4), an effective anticancer drug against several types of solid tumors, including colorectal, breast, pancreatic, renal and lung cancers^[77], causes telomere attrition or shortening in HeLa cells, by increasing the level and stability of TRF2^[99]. By increasing TRF2 expression, gemcitabine enhances the XPF activity, and because XPF is a nuclease, binding of this enzyme to telomeres may lead to inappropriate excision of telomeric DNA. The anticancer effect of gemcitabine is due to the incorporation of the active derivative compound dFdCTP into DNA in proliferating cells, leading to inhibition of DNA synthesis and repair. Thus, the above findings by Su *et al.*^[78] suggest that the promotion of telomere attrition by induction of TRF2 is a new mechanism of action of gemcitabine against cancer. This effect of gemcitabine seems to be independent of telomerase, since this drug had no effect on telomerase activity in HeLa cells 3 days after treatment. No further studies have been made to analyze the effect of gemcitabine on telomere length or telomerase activity in mammalian cells.

C-1027

The enediyne antibiotic C-1027 or Lidamycin (CAS No. 120177-69-7) is a new kind of macromolecular antitumor antibiotics, produced by *Streptomyces globisporus* in soil, consisting of a noncovalently bound apoprotein and a labile chromophore which is responsible for most of the biological activities [79-81]. This drug is a potent anticancer drug with radiomimetic properties, which is being currently evaluated in Phase II clinical trials [82]. Several years ago, it was demonstrated in cultured human colon carcinoma HCT116 cells exposed to C-1027 that this drug induces telomere fusions (i.e., chromosomes joined end to end at their telomeres or fused together after complete loss of telomere sequences, as seen by telomere FISH) in these cells [83]. Therefore, C-1027 induces short-term telomere shortening in human cells. No further studies on the effects of C-1027 on telomere length or telomerase activity have been performed so far.

ICRF-193

ICRF-193 ([meso-2, 3-bis (2, 6-dioxopiperazin-4-yl) butane], CAS No. 21416-68-2) is a topoisomerase II catalytic inhibitor and a well-known anticancer drug for treating acute leukemia and lymphosarcoma [84]. It has been demonstrated that ICRF-193 causes telomere shortening in ALT cells (U2OS and Saos2 human osteosarcoma cell lines) but has no effect on telomere length in telomerase-positive cells (HCT116 human colon cancer cell line and HeLa human cervical cancer cell line) [85]. Moreover, ICRF-193 causes ALT telomere shortening and inhibits ALT cell proliferation in mice [85], which suggests that this drug could be used to prevent cell proliferation in cancer cells with an ALT mechanism of telomere elongation. No further studies on the effects of ICRF-193 on telomere length or telomerase activity have been performed so far.

Melphalan

Melphalan, L-phenylalanine mustard, L-PAM, Alkeran or L-Sarcosine (CAS No. 148-82-3) is a chemotherapeutic drug belonging to the class of nitrogen mustard alkylating agents [86]. It has been reported that melphalan has no effect on telomerase activity in human testicular cancer cells [39]. More recently, by studying the induction and persistence of chromosome aberrations in bone marrow and spleen cells of p53[±] (and wild type) mice exposed for 4, 13, or 26 weeks to 2 mg/kg melphalan, Sgura *et al.* [87] demonstrated that this compound induces telomere shortening in bone marrow cells of wild-type mice, while in p53[±] mice the exposure to this compound induces telomere elongation. No further studies on the effect of melphalan on telomere length or telomerase activity have been reported so far.

5-FU

In the case of 5-fluorouracil (CAS No. 51-21-8), a thymine analog with anticancer properties commonly used against several types of solid tumors, including breast, colorectal, pancreatic, skin and cervical carcinoma [88], there is only a few reports about its effects on telomere length or telomerase activity. It has been shown that 5-FU inhibits telomerase activity in human head and neck squamous cell carcinoma cell lines [52] and, combined with cisplatin, 5-FU increases telomerase activity and causes long-term telomere elongation in colorectal carcinoma cells (LoVo and DLD-1 cell lines) [89].

CPA

Cyclophosphamide (CAS No. 50-18-0) is an alkylating drug with antitumoral properties, clinically used for the treatment of lymphoma, brain cancer, leukemia and some solid tumors, like cancer of the pancreas, and to induce Type 1 and 2 diabetes in laboratory animals [90]. Its clinical use is very limited due to its toxic effects. There are only a few data published concerning the effects of the anticancer drug cyclophosphamide on telomere length and telomerase activity. Decreased telomerase activity and telomere shortening were observed in mouse spermatogonial cells treated with the alkylating compound 4OOH-CPA (a preactivated analog of CPA) [40]. CPA also induced telomere shortening in human ovarian cancer cells, but induced telomerase activity in these cells [44].

Mitomycin C and methotrexate

The chemotherapeutic agents mitomycin C (CAS No. 50-07-7) and methotrexate (formerly amethopterin) (CAS No. 59-05-2) -used for the treatment of several types of cancer, including gastrointestinal cancers, breast cancer, bladder tumors, leukemias, lymphomas and lung cancer- were found to inhibit telomerase activity in human haematopoietic cancer cells and human testicular cancer cells, respectively [39,53].

Telomere length and telomerase activity alterations induced by chemotherapeutic drugs: Conclusions and future prospects.

The studies reviewed here clearly show that telomere length and telomerase activity are differentially affected by exposure to chemotherapeutic drugs. However, from these studies and the analysis of data from Table 1, we can draw some important conclusions:

1) For some chemotherapeutic drugs, their effect on telomere length and/or telomerase activity is clearly established. They induce telomere shortening and/or inhibit telomerase activity. Thus, cisplatin clearly causes telomere shortening and inhibits telomerase activity, paclitaxel induces telomere shortening, and doxorubicin and AZT inhibit telomerase activity. Moreover, streptozotocin do not alter telomerase activity, even in the long-term.

2) For other chemotherapeutic drugs (doxorubicin, 5-AZA, etoposide) contradictory data exist concerning their effects on telomere length and/or telomerase activity, so further studies are needed to clearly determine these effects.

3) For several chemotherapeutic drugs (gemcitabine, C-1027, ICRF-193, melphalan, 5-FU, CPA, mitomycin C, methotrexate) there is only a few data available regarding their effects on telomere length and telomerase activity, so further studies are needed to clearly establish whether they induce telomere shortening or lengthening and whether they induce up- or down-regulation of telomerase activity. Moreover, in the case of bleomycin, streptonigrin, streptozotocin, etoposide and AZT, their effect on telomere length is not clearly established yet.

Despite of the above conclusions, most of the studies performed so far have shown that chemotherapeutic drugs induce telomere shortening and inhibit telomerase activity. Therefore, the data available raise concern about the potential risks of a long-term chemotherapy based on the abovementioned drugs. Telomere shortening and/or telomerase inhibition induced by cytostatic therapy theoretically could induce an additional genomic instability in neoplastic cells, this effect causing undesirable side effects, including secondary malignancies in long-term survivors of cancer.

In summary, for most chemotherapeutic drugs, further studies will be needed to fully elucidate their effects on telomere length and telomerase activity. Depending on the drug, these studies should be aimed at determining whether it induces short- and long-term telomere shortening or elongation, or down- or up-regulation of telomerase activity. Undoubtedly, these studies will contribute to a better understanding of the effects of the anticancer drugs on mammalian cells, and this information will be of great importance to understand the genomic instability associated with chemotherapy regimens.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Abbreviations

TERRA: Telomeric repeat containing RNA; TERT: Telomerase Reverse Transcriptase; TERC: Telomerase RNA Component; ALT: Alternative lengthening of telomeres; CHO: Chinese hamster ovary cells; CHE: Chinese hamster embryo cells; FISH: Fluorescent *in situ* hybridization; TRF: Terminal Restriction Fragment; 4OOH-CPA: Hydroperoxycyclophosphamide; CPA: cyclophosphamide; AZT: 3'-azido-3'-deoxythymidine; 5-FU: 5-Fluorouracil; 5-AZA: 5-azacytidine; XPF: Xeroderma pigmentosum group F protein.

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