

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Telomere Instability Induced by Anticancer Drugs in Mammalian Cells

Alejandro D. Bolzán

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/64928>

Abstract

Telomere instability results from chromosome end loss (due to chromosome breakage at one or both ends) or, more frequently, telomere dysfunction. Dysfunctional telomeres arise when they lose their end-capping function or become critically short, which causes chromosomal termini to behave like a DNA double-strand break. At the chromosomal level, this phenomenon is visualized by using Fluorescence In Situ Hybridization (FISH), as chromosomal aberrations directly involving terminal telomeric repeats: loss or duplication of telomeric signals, association or fusion of telomeres of different chromosomes, telomere sister chromatid exchanges, translocation or amplification of telomeric sequences, and extrachromosomal telomeric signals. At the molecular level, telomere instability arises due to the loss or modification of any of the components of the telomere (telomere DNA, telomere-associated proteins or telomere RNA). Since telomeres play a fundamental role in maintaining genomic stability, the study of telomere instability in cells exposed to anticancer drugs is of great importance to understand the genomic instability associated with chemotherapy regimens. In this chapter, we will summarize our current knowledge about telomere instability induced by anticancer drugs on mammalian cells.

Keywords: Telomere, telomere instability, telomere loss, telomere dysfunction, telomere erosion, chemotherapy, anticancer drugs

1. Introduction

1.1. What are telomeres?

Classically defined as the chromosome ends, telomeres (from the Greek, *telo* = end, and *mere* = part) [1] are nowadays defined as specialized nucleoprotein complexes localized at the physical ends of linear eukaryotic chromosomes, that maintain their stability and integrity [2, 3]. They protect chromosomes from degradation, recombination or fusion, by preventing the

ends of linear chromosomes from being recognized as DNA double-strand breaks (DSB) by the DNA repair machinery, i.e., they distinguish natural DNA ends from DNA ends resulting from breakage events [2, 3].

In all vertebrates, telomeres are composed of tandem arrays of short, repetitive G-rich sequences (TTAGGG)_n, oriented 5' → 3' towards the end of the chromosome, ending in an essential 3' single-stranded overhang that ranges in length from ~50 to 400 nt [4-6], bound by a specialised multiprotein complex known as “shelterin” or telosome [7-9]. The length of the double-stranded telomeric repeat varies greatly among species [2]. In normal human cells, the DNA at each chromosome terminus spans 5-20 kb in length [6, 10, 11], terminating in a 3' single-stranded overhang 100-400 nt in length [10], whereas in human tumor cells, telomere length varies from 1 to 20 kb [12-14].

The telosome is constituted by 6 proteins (POT1, TPP1, TIN2, TRF1, TRF2 and RAP1) and is charged with protecting chromosome ends from activating a DNA damage response, inhibiting inappropriate repair mechanisms, and maintaining telomeric length and structure [7-9]. Besides telomeric repeats and shelterin, telomeres also comprise (UUAGGG)_n-containing RNA molecules (telomeric repeat-containing RNA or TERRA), a novel class of RNA for which several functions have been suggested [15-18]. TERRA transcription occurs at most or all chromosome ends and it is regulated by RNA surveillance factors and in response to changes in telomere length. Therefore, telomeres are composed of DNA, proteins, and RNA. In addition to the shelterin complex, many proteins involved in DNA repair are also associated with telomeres [19].

Telomere length is maintained by a dynamic process of telomere shortening and lengthening. Usually, telomere shortening occurs due to nucleolytic degradation and incomplete DNA replication, due to the inability of lagging strand synthesis to completely replicate chromosomal ends (i.e., the so-called “end replication problem”) [9]. Telomere shortening is usually prevented by telomerase, a reverse transcriptase-like enzyme containing an RNA subunit (TERC, “Telomere RNA Component”) and a catalytic protein subunit called “Telomerase Reverse Transcriptase” (TERT, “Telomere Reverse Transcriptase”) which works via an RNA template -using exclusively single-strand 3' telomeric overhangs as primers- [9], by adding telomeric repeats to the chromosome ends. Although repressed in the majority of normal somatic cells (with the exception of a transient S phase activity thought to maintain the single-stranded overhang), telomerase is present in immortal cell lines, germline cells, stem cells, activated lymphocytes, and most of the tumor cells analyzed so far [20, 21]. Telomerase activity favors 3' overhangs over blunt DNA ends for an addition of telomere sequence, at least *in vitro* [22, 23]. 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 [24].

It has been suggested that TERRA may be involved in the regulation of telomerase activity and that the accumulation of TERRA at telomeres can interfere with telomere replication, leading to telomere loss [15-18]. 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 [25].

The analysis of the short- and long-term chromosomal instability produced by anticancer drugs is of great importance to understand the genomic instability associated with chemotherapy regimens. Since telomeres play a fundamental role in maintaining chromosomal/genomic stability, the study of telomere instability induced by antineoplastic drugs is of clinical interest. Therefore, in this chapter, we will consider in detail the phenomenon of telomere instability and summarize our current knowledge concerning the main data available about telomere instability induced by anticancer drugs on mammalian cells. In the next section, we will see what is telomere instability and how it can be generated in the cells.

2. What is telomere instability? Telomere loss, dysfunction and erosion

Telomere instability refers to the chromosomal instability caused either by the loss of the chromosome ends (one or both) or the dysfunction of telomeres [26, 27]. These phenomena can take place in the short (at first cell division after the induction of chromosome damage by a given mutagen) or in the long term (in the progeny of the exposed cells). Once telomere instability arises, the involved chromosomes tend to associate or fuse with each other [26, 27].

Chromosomal instability is usually defined as a delayed increase in the production of new aberrations or a delayed appearance of excessive levels of aberrations in the clonal surviving cells after exposure to a given mutagen [28]. However, whenever a chromosome lacks one or both of its ends or suffers telomere dysfunction it becomes unstable, so we can refer to chromosome -or more properly, telomere- instability to the one that arises since the first cell division after the chromosome damage event at the telomeric region occurs.

Telomeric instability may arise when a chromosome, due to a break in one or both of its ends, loses one or both telomeres (ie, becomes an "incomplete chromosome") and, therefore, the chromosome end is exposed to enzymatic degradation or fuses with another chromosome end or enters to what is called a breakage-fusion-bridge (BFB) cycle (see below for details). We refer to this type of instability as telomere instability by telomere or chromosome end loss [26, 27, 29]. Alternatively, if one or both telomeres of the chromosome are excessively shortened, the chromosome tends to associate or fuse with other damaged chromosomes. This shortening can affect any of the four telomeres of a (metaphase) chromosome, resulting in duplication or loss of telomeric signals (after Fluorescence In Situ Hybridization or FISH, each FISH signal representing a block or specific set of telomeric sequences) [26, 29]. It may also be the case that any of the telomeric proteins (the shelterin complex) or telomeric RNA be altered or lost. In all these cases, we refer to telomeric instability by telomere dysfunction because the telomere has lost its protective function, either by excessive shortening (termed "telomere erosion or attrition") or by loss or alteration of the proteins or RNA associated with the telomeric DNA [26]. This phenomenon can be studied at the cytogenetic level by telomere FISH and is visible through telomeric chromosomal aberrations such as telomeric fusions, associations or loss or duplication of telomeric signal [26, 29, 30].

2.1. Telomere or chromosome end loss

As previously noted, true telomere loss is due to chromosome breakage at one or both ends of the chromosome, and can generate chromosome instability, both by allowing degradation of the ends of chromosomes and promoting chromosome fusions. Fusion can occur between sister chromatids, or between different chromosomes if telomeres are lost in more than one of the chromosomes of a given cell. Chromosome fusion results in chromosome instability through the abovementioned BFB cycles [26, 27, 29], when chromosomes, after telomere loss, repeatedly fuse and break for many cell generations. BFB cycles can continue for multiple cell generations, leading to extensive chromosomal rearrangements, and terminate when the unstable chromosome eventually acquires a new telomere and so becomes stable [26, 27, 29]. BFB cycles involving sister chromatid fusions result in several types of chromosome rearrangements, including terminal deletions, inverted duplications, DNA amplification, duplicative and nonreciprocal translocations, and dicentric chromosomes, all of which have been associated with human cancer. Chromosomes lacking one telomere remain unstable until they are capped, and lost telomeres after a BFB cycle can be acquired by several mechanisms, including nonreciprocal translocation, duplication/translocation, subtelomeric duplication, or direct telomere addition [26, 27, 29]. For a detailed description of BFB cycles see [26, 27].

2.2. Telomere dysfunction and erosion

As previously stated, dysfunctional telomeres arise when they lose their end-capping function or become critically short (a phenomenon called telomere erosion or attrition), which causes chromosomal termini to behave like a DSB [9, 31]. In effect, dysfunctional (uncapped or shorten) telomeres are sensed as true DSB, according to the presence of DNA damage response proteins at telomeres in senescent cells or shelterin deficient cells [32]. Therefore, dysfunctional telomeres act as DSB, interfering with the correct rejoining of broken ends. Both telomeres and DSB are DNA ends, and as such, both recruit many of the same proteins. As previously mentioned, proteins governing the DNA damage response are intimately involved in the regulation of telomeres, which undergo processing and structural changes that elicit a transient DNA damage response [19]. Chromosomes with dysfunctional telomeres tend to fuse with one another, producing dicentrics, which can give rise to the abovementioned BFB cycles [26, 27]. It must be taken into account that telomere shortening does not always mean telomere dysfunction. Only when telomeric repeats loss gives rise to a defective telomere structure a dysfunctional telomere appears. Thus, telomere erosion refers to a dysfunctional telomere which became critically short, so it cannot function properly.

Telomere dysfunction at the chromosomal level is commonly assessed applying the telomere FISH technique to metaphase chromosomes [26, 29, 30]. A normal metaphase chromosome exhibits four telomeric signals, two at each end (one per chromatid). When the telomere becomes dysfunctional, one or more of these telomeric signals are lost or duplicated [26, 29, 30]. The presence of chromosome ends with undetectable telomeric hybridization signals has been shown to be a good indicator of critically short and probably dysfunctional telomeres in mammalian cells [33-36]. It is important to mention that not all telomere involving chromosomal aberrations imply telomere dysfunction, but only those ones directly involving terminal telomeric repeats (see [26, 29] for details).

Telomeres suppress the DNA damaging response, so dysfunctional telomeres activate DNA damage checkpoints [19, 32]. In addition, dysfunctional telomeres induce metabolic and mitochondrial compromise [37], promote carcinogenesis [38, 39], induce chromosome instability [27], and triggers cellular senescence [40].

3. Factors promoting telomere instability

Several factors can promote telomere instability. Telomere instability due to true telomere loss can be generated by any mutagen which breaks the chromosome and induces terminal deletions, as shown by several studies (see [26] for review). This kind of instability gives rise to the so-called “incomplete chromosome elements”, which comprise chromosomes without one or both telomeres (incomplete chromosomes) and the acentric fragments resulting from the breakage event (termed “terminal fragments”) [26, 29, 30].

Telomere instability due to telomere dysfunction can be generated in several ways [26]. Alterations in the shelterin complex or other telomere-binding proteins [7, 8, 41], some DNA damage response proteins required for proper telomere protection [42], the structure of telomeric DNA (loss of telomeric sequences, see below), the structure or activity of telomerase [43], TERRA [15-18] or the enzymes helicases [44, 45] can give rise to dysfunctional telomeres. All these factors are involved in the production of telomere-related chromosomal aberrations. These aberrations have been described in detail elsewhere [26, 29, 30] and thus they will not be considered in the present chapter. Moreover, dysfunctional telomeres may result as a consequence of mutagen-induced telomeric DNA damage [26].

Referring to the relationship between telomere shortening and dysfunction, we must bear in mind that, as previously mentioned, telomere length is maintained by a dynamic process of telomere shortening and lengthening. Telomeres lose approximately 20-300 bp of repeat sequences every cell division mainly due to the “end replication problem” [9]. This is the most obvious mechanism for the loss of telomeric repeat sequences, i.e., attrition due to the failure to compensate for the gradual loss of these repeats during cell division, and is termed replicative erosion or replicative shortening, which leads to replicative senescence of cells. Thus, telomeres regulate the replicative life span of somatic cells, acting as a “mitotic clock”. There is another kind of telomere shortening, termed “stress dependant shortening”, which is produced by stress-inducing factors like radiation, oncogenes, oxidative damage within telomeric DNA, chromosome end-specific exonuclease activity, and the lack of telomerase activity [46-49]. Stress dependant shortening can lead to the loss of large blocks of telomeric repeat sequences through different mechanisms, including recombination, problems encountered during DNA synthesis or inefficient DNA repair. In addition, telomere shortening is accelerated by active oxygen species and ultraviolet radiation, which are thought to be major environmental causes of human telomere shortening [46]. In the next section, we will summarize our current knowledge concerning the main data available about telomere instability induced by anticancer drugs on mammalian cells.

4. Telomere instability produced by anticancer drugs in mammalian cells

In the next sections, we will consider the main data available concerning the short- and long-term telomere instability induced by anticancer drugs in mammalian cells. Firstly, we will refer to those drugs whose effects on telomeres have been intensively investigated: bleomycin, streptonigrin, streptozotocin, paclitaxel, cisplatin, doxorubicin, etoposide and 5-azacytidine. Afterwards, we will shortly refer to other anticancer drugs whose effects on telomeres are barely known, such as gemcitabine, C-1027, ICRF-193, melphalan and 5-fluorouracil. It is important to bear in mind that, in this chapter, when we refer to telomeres, we refer to the very end of the chromosomes (which, at the molecular level is constituted by TTAGGG repeats and the associated RNA and proteins), not the subtelomeric region of them (constituted by telomere-specific sequences located near the telomere). Therefore, we will not refer to those studies involving the effects of anticancer drugs on the subtelomeric regions of the chromosomes.

4.1. Bleomycin (BLM), Streptonigrin (SN) and Streptozotocin (STZ)

Several years ago, we carried out in our laboratory a series of experiments to determine the short-term effects of three antibiotics with anticancer properties, bleomycin (BLM), streptonigrin (SN) and streptozotocin (STZ) on mammalian telomeres and telomeric sequences (see [26] for review).

BLM (CAS No. 11056-06-7) is a chemotherapeutic drug isolated from *Streptomyces verticillus* which is commonly used to treat testicular cancer, lymphoma, lung cancer, cervical cancer and cancers of the head and neck [50]. This antibiotic is an S-independent clastogen and a radiomimetic agent that generates free radicals and induces single- and double-strand breaks in DNA [50, 51]. SN (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 [52], although its use in cancer therapy is very limited because it induces severe and prolonged bone marrow depression [52]. Despite of being considered a radiomimetic compound, SN is capable of producing chromosome damage both by S-independent and S-dependent mechanisms [52]. Moreover, SN causes inhibition of topoisomerase II by stabilizing the transesterification intermediate of the enzyme (called cleavable complex) [52]. STZ (CAS No. 18883-66-4) is an antibiotic isolated from *Streptomyces achromogenes* [53, 54], usually used to experimentally induce diabetes mellitus in laboratory animals, and it has been considered a potential compound for the clinical treatment of some malignant diseases, including advanced pancreatic neuroendocrine tumors and colon cancer. STZ is a potent alkylating agent that directly methylates DNA, giving rise to chromosome and DNA damage [53, 54]. STZ exerts its clastogenic effect mainly in an S-dependent manner, inducing both chromatid- and chromosome-type aberrations [53, 54].

The abovementioned studies, performed using FISH with a Peptide Nucleic Acid telomere probe (telomere PNA-FISH) in Chinese hamster cells (CHE cell line), showed that all the above drugs can induce the formation of incomplete chromosomes and terminal fragments [55-58].

These observations were made on metaphase cells obtained 18 h after treatment (i.e., in cells in their first mitosis after treatment) and indicated that, despite of the fact that BLM, SN, and STZ act on chromosomes in a different way, these drugs can induce short-term telomere instability by chromosome end loss in mammalian cells. The induction of short-term telomere instability by BLM was also demonstrated by Benkhaled et al. [59] in human lymphocytes. More recently, our studies were focused on the effects of these antibiotics on the progeny of the exposed cells, to determine if telomeres play some role in the long-term chromosomal instability induced by these drugs and if telomere instability can persist in the exposed cells for several generations after treatment. To accomplish our goal, we exposed rat cells (ADIPO-P2 cell line) to a single pulse of BLM, SN or STZ, and determined the type and frequency of chromosomal aberrations at 18 h (first mitosis after exposure), 10 days and 15 days after treatment by using PNA-FISH with a telomeric probe.

We found that BLM induces persistent telomere instability in mammalian cells, cytogenetically manifested as incomplete chromosome elements (i.e., chromosome end loss) and telomere FISH signal loss and duplication (i.e., telomere dysfunction) ([60] and Paviolo, unpublished data). In addition, our results suggested that BLM can induce delayed telomere instability in the form of telomere (end-to-end) fusions. Therefore, we concluded that BLM induces telomere instability at the chromosome level both by chromosome end loss and telomere dysfunction [60]. The delayed appearance of dicentric chromosomes and telomere fusions (which produces dicentric chromosomes without accompanying fragment) that we observed in ADIPO-P2 cells exposed to BLM suggests that the BFB cycles [27] might play a significant role in the maintenance of the long-term telomere instability induced by this compound. In effect, by inducing breakage at terminal regions of chromosomes, resulting in incomplete chromosomes, BLM could promote genome instability through BFB cycles, which can continue for multiple cell generations, leading to extensive chromosomal rearrangements in the progeny of the cells exposed to this compound. According to our data, the persistent telomere instability induced by BLM in rat cells is neither related to telomerase activity nor telomere length variations ([60] and Paviolo, unpublished data).

In the case of SN, we found that this drug induces persistent (i.e., up to 15 days after treatment) telomere dysfunction in ADIPO-P2 cells in the form of additional telomeric FISH signals, extrachromosomal telomeric FISH signals, and telomere FISH signal loss and duplications [61]. Several studies have provided a large body of evidence indicating that SN directly interacts with DNA, binding covalently but not interacting into the double helix (see [52] for review). Therefore, the persistence of the clastogenic action of SN in terms of telomere-associated aberrations could be due to the formation of a stable complex between SN and the DNA molecule, which may induce chromosome damage through a persistent cyclic redox process and the resulting generation of active oxygen species [52]. Moreover, we found that SN causes persistent inhibition of telomerase activity in rat cells [61]. A decreased telomerase activity could promote extensive telomere shortening, cytogenetically detected as telomere FISH signal loss. However, using the Flow-FISH technique, we were able to determine that SN does not have a persistent effect on telomere length in rat cells, since we observed a transient telomere lengthening in these cells only at 10 days after treatment (Paviolo, unpublished data). Therefore, the precise relationship between telomerase activity, telomere length and telomere instability in SN-exposed cells remains to be determined.

It is interesting to note that despite of the fact that both BLM and SN are radiomimetic compounds, they exhibit some important differences in their long-term effects on telomeres [60, 61]. First, BLM induces persistent chromosome end loss and telomere dysfunction, whereas SN induces persistent telomere dysfunction but not persistent chromosome end loss. Second, BLM induces telomere fusions, whereas SN not. Finally, BLM induces delayed increase of telomerase activity in mammalian cells, while SN decreases telomerase activity. Although these discrepancies could be due to differences between SN and BLM in their mode of action [50-52], further studies will be needed to confirm this assumption.

Finally, we found that also STZ induces persistent telomere dysfunction in rat cells, cytogenetically detected mainly as telomere FISH signal loss and duplications, most of them being chromatid-type aberrations [62]. We observed that STZ induces significantly more signal loss than duplications, telomere loss thus being the most significant effect of STZ on telomere function at the chromosome level in ADIPO-P2 cells. As previously mentioned, telomere FISH signal loss and duplication were also observed in BLM- [60] and SN-exposed ADIPO-P2 cells [61]. Therefore, these types of aberrations seem to be the predominant chromosome aberrations directly related to telomere dysfunction induced by anticancer drugs. In addition, our experiments with rat cells exposed to STZ showed that this compound also induces long-term telomere instability in the form of incomplete chromosome elements, as previously observed in the short-term in Chinese hamster cells [57]. We found that STZ induces a transient increase in telomere length in ADIPO-P2 cells at 10 days after treatment, a delayed effect not related with telomerase activity, which remained unchanged in both treated and untreated cells [62]. Therefore, the persistence of chromosomal aberrations related to telomere dysfunction in rat cells exposed to STZ seems to be unrelated to telomerase activity or telomere length.

Besides the abovementioned studies, other researchers reported additional data on the effect of BLM on telomeres. No further studies have been made concerning the effects of SN and STZ on telomeres so far. The most important finding with regard to BLM was that human telomeric DNA sequences are a major target for this anticancer drug [63, 64]. In effect, these authors examined the DNA sequence specificity of BLM in a target DNA sequence containing 17 repeats of the human telomeric sequence and other primary sites of BLM cleavage and found that BLM cleaved primarily at 5'-GT in the telomeric sequence 5'-GGGTTA [63, 64]. The telomeric region constituted 57% of the 30 most intense BLM damage sites in the DNA sequence examined, these data indicating that telomeric DNA sequences are a major target for BLM damage. Previously, by using the Comet-FISH technique (i.e., single cell gel electrophoresis or Comet assay in combination with Fluorescent in situ hybridization), with a telomere-specific PNA probe, Arutyunyan et al. [65] found that BLM and Mitomycin C (MMC, CAS No. 50-07-7) induce breaks in telomere-associated DNA in human lymphocytes. The breakage frequency for telomeric DNA was found to be proportional to that of the total DNA, which suggests random induction of DNA breaks by these drugs. A year later, these authors using the same technique showed that, in human lymphocytes, BLM and also the anticancer drug cisplatin induce telomere DNA damage [66]. The action of cisplatin on telomeres will be considered in detail later in this chapter.

The induction of telomere DNA damage by BLM (and also MMC) in mammalian cells was confirmed by Hovhannisyan et al. [67], who analyzed the effect of these drugs in normal human leukocytes and three transformed cell lines (HT1080, CCRF-CEM and CHO) using the Comet-

FISH assay. They found significant differences between these cells with respect to quantitative head/tail distribution of telomeric signals after BLM exposure, which indicates that the extent of the telomere DNA damage induced by this compound depends on the cell type. Recently, Liu et al., studied the effect of BLM and other anticancer drugs on telomeres of a mouse spermatogonial cell line and found that BLM damages telomeric DNA (as seen by the co-localization of telomere and gamma-H2AX signals after FISH and immunofluorescence) [68].

4.2. 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 a wide range of human cancers, including ovarian, breast, lung and pancreatic cancers [69]. The anticancer effect of paclitaxel is attributable principally to irreversible promotion of microtubule stabilization and is hampered upon development of chemoresistance by tumor cells [70, 71].

It has been shown that paclitaxel and water-soluble poly (L-glutamic acid)-paclitaxel induce telomeric associations in a murine metastatic melanoma cell line (K1735, clone X-21), being the effect of the water-soluble form of paclitaxel more pronounced than the effect of paclitaxel alone [72]. Two years later, these authors analyzed the effects of the above compounds and two other water-soluble forms of paclitaxel (sodium-pentetic acid-paclitaxel and polyethylene glycol-paclitaxel) in the same murine cell line and found that these drugs induce the formation of telomeric associations [73]. In addition, they found that paclitaxel and its water-soluble conjugates induce extensive telomere erosion (visualized as reduced telomeric signal intensity after telomere FISH) but do not change telomerase activity [73]. Therefore, these drugs induce telomere dysfunction in mammalian cells by producing telomeric associations and telomere erosion (which means loss of telomeric repeats). Telomeric associations and reduction of telomeric signal intensity were also observed in Tax-18 and Tax-2-4, two paclitaxel-requiring mutant Chinese hamster ovary (CHO) cell lines [74]. Moreover, in these cells, cell death was driven by the loss of telomeric DNA repeats, as shown by the analysis of terminal telomeric restriction fragments [74]. Telomere erosion induced by paclitaxel can be enhanced by telomerase inhibitors, such as 3'-azido-3'-deoxythymidine (AZT) [75, 76]. More recently, using telomerase-deficient cells derived from mTERC^{-/-} (mouse telomerase RNA component-minus) mice, Park et al. demonstrated that, upon telomere erosion, paclitaxel stimulates chromosomal fusion and instability in cells with dysfunctional telomeres [77]. Chromosomal fusions promoted by paclitaxel involve both q- and p-chromosome arms, being the q-arm fusions both unstable and lethal [77]. These chromosomal fusions occur in response to microtubule disruption induced by paclitaxel in cells with dysfunctional telomeres [77]. Thus, telomere dysfunction, rather than telomerase inhibition seems to be essential to sensitize transformed cells to paclitaxel.

4.3. Cisplatin

Cisplatin (CAS No. 15663-27-1), another well-known anticancer drug, was also found to interact with telomeric DNA sequences. Ishibashi and Lippard [78] showed that cisplatin can bind to telomeric repeats: Duplex DNA containing five telomeric repeats treated with cisplatin

at formal platinum/strand ratios of 5 or 10 in water was platinated with efficiencies of 91.0% and 76.4%, respectively. More recently, Paul and Murray, using an automated capillary DNA sequencer investigated the interaction of cisplatin with purified telomeric DNA sequences and found that cisplatin strongly formed adducts with telomeric DNA sequences [79]. A similar result was obtained by Murray and Kandasamy, using a plasmid clone containing seven telomeric repeats and a sequence of ten consecutive guanine bases [80]. Although cisplatin preferentially damaged the guanine sequence, the telomeric DNA was also a major site of cisplatin adduct formation [80]. Furthermore, Nguyen et al. analyzed the DNA sequence specificity of cisplatin in a long telomeric tandem repeat (a human telomeric DNA sequence containing 17 tandem repeats) and found that the 3'-end of the G-rich strand of the telomeric repeat was preferentially damaged by this compound [81].

Even though several studies showed that cisplatin inhibits telomerase activity in a specific and concentration-dependent manner in several types of cancer cells (see [82, 83] for example), little is known about whether this compound induces telomere instability. Ishibashi and Lippard [78] by using Analysis of Terminal Restriction Fragment (TRF) Length (by Southern blot) showed that cisplatin induces telomere loss (shortening) and degradation in HeLa cells. A recent study from Liu et al. in a mouse spermatogonial cell line, showed that the alkylating compounds cisplatin and 4-hydroperoxycyclophosphamide (4OOH-CPA, a preactivated analog of cyclophosphamide, CAS No. 50-18-0) induce telomere dysfunction in mouse cells [68]. These authors found that these compounds decrease telomerase activity and shorten telomere length, thus causing telomere dysfunction [68]. Thus, cisplatin and 4OOH-CPA could induce long-term telomeric loss in mammalian cells, resulting from the inhibition of the enzyme telomerase.

4.4. 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. As is the case with BLM and cisplatin, doxorubicin can also bind to human telomeric repeats. In effect, it was demonstrated that this drug binds to the human telomeric sequence 5'-d[GGG(TTAGGG)(3)]-3' (21-mer), assuming a G-quadruplex structure in the presence of K(+) [84]. It has been found that doxorubicin inhibits telomerase activity and shortens mean telomere length in human hepatoma cells [85]. 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 showed no effect on telomerase or telomere length in human ovarian cancer cells [86], induces telomere dysfunction (as determined by the presence of end-to-end chromosome fusions and end breaks by conventional staining with Giemsa, not telomere FISH) and decreases telomerase activity but has no effect on telomere length in breast tumor cells [87], and decreases telomerase activity in several human breast and stomach cancer cell lines [88]. Thus, the effect of doxorubicin on telomeres depends on the cell type. Moreover, it has been shown that doxorubicin induces senescence or apoptosis in rat neonatal cardiomyocytes by regulating the expression levels of the telomere binding factors 1 and 2: High-dose doxorubicin strongly reduces TRF2 expression while enhancing TRF1 expression, and it

determines early apoptosis, whereas low-dose doxorubicin induces downregulation of both TRF2 and TRF1 [89]. The exposed cells maintain telomere dysfunction and a senescent phenotype over time and undergo late death. Therefore, this study suggests that doxorubicin induces telomere dysfunction at the molecular level by regulating the expression levels of TRF1 and TRF2, both of them being part of the shelterin complex. A few years ago, it was reported that doxorubicin and etoposide induce progressive telomere shortening (assessed by flow-fluorescence in situ hybridization and Southern blotting) in human mesenchymal stem cells (MSCs), obtained from bone marrow (BM) cells from normal adults and grown in the presence of platelet lysates [90]. 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 telomerase reverse transcriptase (hTERT) and the telomere binding proteins TPP1 and POT1 and telomere dysfunction in these cells [91]. Therefore, both topoisomerase II poisons doxorubicin and etoposide, induce telomere dysfunction. 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 [68], but in combination with BLM and cisplatin, etoposide produces telomere shortening in rat male germ cells [92]. In addition, it was demonstrated that etoposide did not affect telomere length in the neuroblastoma cell line SHSY5Y, with very short telomeres and the acute lymphoblastic T cell line 1301, which displays extremely long telomeres [93]. Thus, the effect of etoposide on telomeres depends on the cell type.

4.5. 5-azacytidine (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 [94]. It has been shown that 5-aza-2'-deoxycytidine, either alone or in combination with trichostatin A, induces up-regulation of shelterin genes, which leads to telomere elongation in breast cancer cell lines [95]. In addition, 5-AZA was found to induce DNA damage at telomeres and telomere dysfunction in acute myeloid leukemia cell lines [96]. Telomere dysfunction was coupled with telomere shortening, diminished TERT expression and apoptosis in the exposed cells [96]. Thus, these authors suggested that another mechanism (besides DNA demethylation) by which 5-AZA exerts its anticancer activity is telomere dysfunction [96]. 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 [97]. Thus, further studies will be needed to clarify the effect of this compound on telomeres.

4.6. 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 cancer [98], causes telomere attrition or shortening in Hela cells, by increasing the level and stability of TRF2 [99], that is required for

the Xeroderma pigmentosum group F protein (XPF)-dependent telomere loss or degradation. By increasing TRF2 expression, gemcitabine enhances XPF activity, and because XPF is a nuclease, binding of the nuclease 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. [99] 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 mammalian telomeres.

4.7. 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 [100-102]. This drug is a potent anticancer drug with radiomimetic properties, which is being currently evaluated in Phase II clinical trials [103]. 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) in these cells [104]. Therefore, C-1027 induces short-term telomere dysfunction in human cells. No further studies on the effects of C-1027 on telomere stability have been performed so far.

4.8. 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 [105]. Two recent publications deal with the effect of ICRF-193 on telomeres [106, 107]. These studies show that this drug induces DNA damage at telomeres (as assessed by colocalization of telomere PNA-FISH signals and immunofluorescence of 53BP1 foci) [106] and telomere dysfunction in the HT1080 fibrosarcoma cell lines [106] and telomere shortening in mice cells [107]. In particular, it was found that ICRF-193 induces damage at telomeres properly capped by TRF2 but not by POT1 [106]. Moreover, ICRF-193 treatment blocks ALT-associated phenotypes in vitro and inhibits ALT cell proliferation in mice [107], 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 stability have been performed so far.

4.9. Melphalan

Melphalan, L-phenylalanine mustard, L-PAM, Alkeran or L-Sarcolysine (CAS No. 148-82-3) is a chemotherapeutic drug belonging to the class of nitrogen mustard alkylating agents [108]. It has been reported that melphalan has no effect on telomerase activity in human testicular cancer cells [82]. 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 (MLP), Sgura et al. [109] were able to demonstrate that this

drug 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 telomeres have been reported so far.

4.10. 5-fluorouracil (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 [110], there is no information about its effects on telomeres as a single drug, since this compound is usually employed in chemotherapy in combination with several drugs. Thus, for example, it has been reported that, combined with cisplatin, 5-FU increases telomerase activity and causes long-term telomere elongation in colorectal carcinoma cells (LoVo and DLD-1 cell lines) [111]. It is interesting to mention that it has been recently demonstrated that overexpression of a human telomerase reverse transcriptase polypeptide (hTERT)C27, which induces telomere dysfunction by promoting end-to-end chromosome fusions, sensitizes HeLa cells and nasopharyngeal carcinoma cells to 5-FU cytotoxic effects [112, 113]. Thus, despite the fact that 5-FU does not induce telomere dysfunction, these recent studies suggest that combinational therapy of this drug with hTERTC27 may provide a novel approach to treat cancer.

5. Telomere instability induced by anticancer drugs: Conclusions and future prospects

Even though the studies performed so far demonstrate that a lot of work needs to be done in order to fully understand the effects of anticancer drugs on telomere stability and function, some important conclusions can be drawn from these studies.

1. For some drugs (BLM, SN. STZ, paclitaxel, 5-azacytidine, etc.) it is clearly established that they induce telomere instability (either by chromosome end loss or telomere dysfunction) in the short- and/or the long-term in mammalian cells, whereas for most of the anticancer drugs this effect remains to be determined. In fact, the literature reviewed in this chapter clearly shows that there are very few studies concerning the long-term effect of anticancer drugs on telomeres. Most of these studies were performed in the last few years. Moreover, little is currently known about the effect of several chemotherapeutic agents (for example, cyclophosphamide, mitomycin C and melphalan) on mammalian telomere dynamics, including potential effects on telomere length, structure, function, telomerase activity, and telomere shelterin proteins.
2. For some drugs (for example, 5-fluorouracil) it remains to be determined if they induce telomere instability per se, since the studies performed so far were carried out using these drugs in combination with other drugs, but not in a single form.
3. For several anticancer drugs (i.e., doxorubicin, etoposide, 5-azacytidine), published data on the effects of telomeres are contradictory. Thus, additional studies will be needed to clarify this issue.

4. Some drugs (i.e., 5-azacytidine and trichostatin A) could exert its anticancer effect by inducing telomere lengthening instead of shortening or erosion. Telomere elongation induced by these drugs may have the effect of stabilizing the telomere thus reducing the amount of genetic damage the cell will undergo, thereby stopping the clonal evolution of the cancer cell population. As a result, these tumors may be more susceptible to further treatment. Alternatively, the elongation of telomeres in cancer cells may give rise to chemoresistant tumors.
5. Some anticancer drugs -like paclitaxel- could exert their anticancer effect by inducing telomere fusions. Thus, this drug could be used to enhance chemosensitivity in cells with dysfunctional telomeres.
6. Finally, regardless of the underlying mechanism involved in the long-term telomere instability induced by some anticancer drugs, the data available raise concern about the potential risks of a long-term chemotherapy based on these drugs. Damage to telomeres induced by cytostatic therapy theoretically could generate telomere shortening and, subsequently, 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, further studies will be needed to fully elucidate the effects of anticancer drugs on telomere stability. Depending on the drug, these studies should be aimed at determining whether it induces short- and long-term telomere instability or not, and if this telomere instability is due to chromosome breakage or telomere dysfunction. Undoubtedly, these studies will contribute to a better understanding of the effects of the anticancer drugs on mammalian telomeres. This information will be of great importance to understand the genomic instability associated with chemotherapy regimens.

Acknowledgements

The author acknowledges the financial support of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, grant PIP No. 0182), Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), and University of La Plata (UNLP) from Argentina.

Author details

Alejandro D. Bolzán*

Address all correspondence to: abolzan@imbice.gov.ar; adbolzan64@gmail.com

Laboratorio de Citogenética y Mutagénesis, Instituto Multidisciplinario de Biología Celular (IMBICE, CCT-CONICET La Plata – CICPBA-UNLP), La Plata, Argentina

References

- [1] Muller HJ: The remaking of chromosomes. *Collecting Net.* 1938;13:181-198.
- [2] Zakian VA: Telomeres: beginning to understand the end. *Science* 1995;270(5242): 1601-1607. DOI: 10.1126/science.270.5242.1601
- [3] Blackburn EH: Switching and signaling at the telomere. *Cell* 2001;106(6):661-673. DOI:10.1016/S0092-8674(01)00492-5
- [4] Meyne J, Ratliff RL, Moyzis RK: Conservation of the human telomere sequence (TTAGGG)_n among vertebrates. *Proc. Natl. Acad. Sci. USA* 1989;86(18):7049-7053
- [5] Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG et al.: Distribution of nontelomeric sites of (TTAGGG)_n telomeric sequences in vertebrate chromosomes. *Chromosoma* 1990;99(1):3-10.
- [6] Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD et al.: A highly conserved repetitive DNA sequence, (TTAGGG)_n, present at the telomeres of human chromosomes. *Proc. Natl. Acad. Sci. USA* 1988;85(18):6622-6626.
- [7] Schmutz I, de Lange, T: Shelterin. *Current Biology* 2016; 26(10):R397-399. DOI: 10.1016/j.cub.2016.01.056
- [8] Palm W, de Lange T: How shelterin protects mammalian telomeres. *Annu. Rev. Genet.* 2008;42:301-334. DOI: 10.1146/annurev.genet.41.110306.130350
- [9] O'Sullivan RJ, Karlseder J: Telomeres: protecting chromosomes against genome instability. *Nat. Rev. Mol. Cell Biol.* 2010;11(3):171-181. DOI: 10.1038/nrm2848
- [10] 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): 2801-2809.
- [11] Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB et al.: Telomere length predicts replicative capacity of human fibroblasts. *Proc. Natl. Acad. Sci. U S A.* 1992;89(21):10114-10118.
- [12] Schmitt H, Blin N, Zankl H, Scherthan H: Telomere length variation in normal and malignant human tissues. *Genes Chromosom. Cancer* 1994;11(3):171-177.
- [13] Bolzán AD, Páez GL, Bianchi MS, Bianchi NO: Analysis of telomeric repeats and telomerase activity in human colon carcinoma cells with gene amplification. *Cancer Genet. Cytogenet.* 2000;120(2):166-170. DOI: 10.1016/S0165-4608(00)00209-0
- [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):2011-2015. DOI: 10.1126/science.7605428

- [15] Azzalin CM, Lingner J: Telomeres: the silence is broken. *Cell Cycle* 2008;7(9): 1161-1165. DOI:10.4161/cc.7.9.5836
- [16] Luke B, Lingner J: TERRA: telomeric repeat-containing RNA. *EMBO J.* 2009;28(17): 2503-2510. DOI: 10.1038/emboj
- [17] Feuerhahn S, Iglesias N, Panza A, Porro A, Lingner J: TERRA biogenesis, turnover and implications for function. *FEBS Lett.* 2010;584(17):3812-3818. DOI: 10.1016/j.febslet
- [18] Cusanelli E, Chartrand P: Telomeric repeat-containing RNA TERRA: a noncoding RNA connecting telomere biology to genome integrity. *Front. Genet.* 2015;6(143):1-9. DOI: 10.3389/fgene
- [19] Arnoult N, Karlseder J: Complex interactions between the DNA-damage response and mammalian telomeres. *Nat. Struct. Mol. Biol.* 2015;22(11):859-866. DOI: 10.1038/nsmb.3092
- [20] Artandi SE, DePinho RA: Telomeres and telomerase in cancer. *Carcinogenesis* 2010;31(1):9-18. DOI: 10.1093/carcin/bgp268
- [21] Chiodi I, Belgiovine C, Mondello C: Telomerase and telomeric proteins: A life beyond telomeres. In A.N. Gagnon, editor. *Telomerase: Composition, Functions and Clinical Implications*. 1st ed. New York: Nova Science; 2010 p. 35-58
- [22] Blackburn EH: Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett.* 2005;579(4):859-862. DOI: 10.1016/j.febslet.2004.11.036
- [23] Chan SR, Blackburn EH: Telomeres and telomerase. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 2004;359(1441):109-121. DOI: 10.1098/rstb.2003.1370
- [24] Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB et al.: Telomerase maintains telomere structure in normal human cells. *Cell* 2003;114(2):241-253. DOI: 10.1016/S0092-8674(03)00550-6
- [25] Draskovic I, Londono-Vallejo A: Telomere recombination and the ALT pathway: a therapeutic perspective for cancer. *Curr Pharm Des.* 2014;20(41):6466-6471. DOI: 10.2174/1381612820666140630085857
- [26] Bolzán AD: Chromosomal aberrations involving telomeres and interstitial telomeric sequences. *Mutagenesis* 2012;27(1):1-15. DOI: 10.1093/mutage/ger052
- [27] Murnane JP: Telomere dysfunction and chromosome instability. *Mutat. Res.* 2012;730(1-2):28-36. DOI: 10.1016/j.mrfmmm.2011.04.008
- [28] Bouffler SD, Morgan WF, Pandita TK, Slijepcevic P: The involvement of telomeric sequences in chromosomal aberrations. *Mutat. Res.* 1996;366(2):129-135. DOI: 10.1016/S0165-1110(96)90033-0

- [29] Bolzán AD: Cytogenetic evaluation of telomere dysfunction: Chromosomal aberrations involving telomeres and interstitial telomeric sequences. In: L. Mancini, editor. *Telomeres: Function, Shortening and Lengthening*. 1st ed. New York: Nova Science Publishers Inc.; 2009 p. 133-185.
- [30] Bolzán AD, Bianchi MS: Telomeres, interstitial telomeric repeat sequences, and chromosomal aberrations. *Mutat. Res.* 2006;612(3):189-214. DOI: 10.1016/j.mrrev.2005.12.003
- [31] Bailey SM, Cornforth MN: Telomeres and DNA double-strand breaks: ever the twain shall meet? *Cell. Mol. Life Sci.* 2007;64(22):2956-2964. DOI: 10.1007/s00018-007-7242-4
- [32] Denchi EL: Give me a break: How telomeres suppress the DNA damage response. *DNA Repair* 2009; 8(9):1118-1126. DOI: 10.1016/j.dnarep.2009.04.013
- [33] Hemann MT, Strong MA, Hao LY, Greider CW: The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* 2001;107(1):67-77. DOI: 10.1016/S0092-8674(01)00504-9
- [34] Espejel S, Franco S, Rodríguez-Perales S, Bouffler SD, Cigudosa JC, Blasco MA: Mammalian Ku86 mediates chromosomal fusions and apoptosis caused by critically short telomeres. *EMBO J.* 2002;21(9): 2207-2219. DOI: 10.1093/emboj/21.9.2207
- [35] Deng W, Tsao SW, Guan XY, Lucas JN, Si HX, Leung CS et al.: Distinct profiles of critically short telomeres are a key determinant of different chromosome aberrations in immortalized human cells: whole-genome evidence from multiple cell lines. *Oncogene* 2004;23(56):9090-9101. DOI: 10.1038/sj.onc.1208119
- [36] Soler D, Genesca A, Arnedo G, Egozcue J, Tusell L: Telomere dysfunction drives chromosomal instability in human mammary epithelial cells. *Genes Chromosomes Cancer* 2005;44(4):339-350. DOI: 10.1002/gcc.20244
- [37] Sahin E, Colla S, Liesa M, Moslehi J, Müller FL, Guo M et al.: Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature* 2011;470(7334):359-365. DOI: 10.1038/nature09787
- [38] Bojovic B, Crowe DL: Telomere dysfunction promotes metastasis in a TERC null mouse model of head and neck cancer. *Mol. Cancer Res.* 2011;9(7):901-913. DOI: 10.1158/1541-7786.MCR-10-0345
- [39] Ma H, Zhou Z, Wei S, Liu Z, Pooley KA, Dunning AM et al.: Shortened telomere length is associated with increased risk of cancer: a meta-analysis. *PLoS One* 2011;6(6):e20466. DOI: 10.1371/journal.pone.0020466
- [40] Cao K, Blair CD, Faddah DA, Kieckhafer JE, Olive M, Erdos MR et al.: Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. *J. Clin. Invest.* 2011; 121(7):2833-2844. DOI: 10.1172/JCI43578

- [41] Donate LE, Blasco MA: Telomeres in cancer and ageing. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2011; 366(1561):76-84. DOI: 10.1098/rstb.2010.0291
- [42] Raynaud CM, Sabatier L, Philipot O, Olaussen KA, Soria JC: Telomere length, telomeric proteins and genomic instability during the multistep carcinogenic process. *Critical Rev. Oncol. Hematol.* 2008;66(2):99-117. DOI: 10.1016/j.critrevonc.2007.11.006
- [43] Stohr BA, Xu L, Blackburn EH: The terminal telomeric DNA sequence determines the mechanism of dysfunctional telomere fusion. *Mol. Cell* 2010;39(2):307-314. DOI: 10.1016/j.molcel.2010.06.020.
- [44] Chavez A, Tsou AM, Johnson FB: Telomeres do the (un)twist: helicase actions at chromosome termini. *Biochim. Biophys. Acta* 2009;1792(4):329-340. DOI: 10.1016/j.bbadis.2009.02.008
- [45] Paeschke K, McDonald KR, Zakian VA: Telomeres: structures in need of unwinding. *FEBS Lett.* 2010; 584(17):3760-3772. DOI: 10.1016/j.febslet.2010.07.007
- [46] 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):186-193. DOI: 10.1006/excr.1995.1305
- [47] Kawanishi S, Oikawa S: Mechanism of telomere shortening by oxidative stress. *Ann. N. Y. Acad. Sci.* 2004;1019:278-284. DOI: 10.1196/annals.1297.047
- [48] Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA et al.: Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 1997;91(1):25-34. DOI: 10.1016/S0092-8674(01)80006-4
- [49] Ayoubaz A, Raynaud C, Heride C, Revaud D, Sabatier L: Telomeres: Hallmarks of radiosensitivity. *Biochimie* 2008;90(1):60-72. DOI: 10.1016/j.biochi.2007.09.011
- [50] Chen J, Stubbe J: Bleomycins: towards better therapeutics. *Nat. Rev. Cancer* 2005;5(2):102-112. DOI: 10.1038/nrc1547
- [51] Povirk LF, Austin MJ: Genotoxicity of bleomycin. *Mutat. Res.* 1991;257(2):127-143. DOI: 10.1016/0165-1110(91)90022-N
- [52] Bolzán AD, Bianchi MS: Genotoxicity of streptonigrin: a review. *Mutat. Res.* 2001;488(1):25-37. DOI: 10.1016/S1383-5742(00)00062-4
- [53] Bolzán AD, Bianchi MS: Genotoxicity of streptozotocin. *Mutat. Res.* 2002;512(2-3):121-134. DOI: 10.1016/S1383-5742(02)00044-3
- [54] Bolzán AD: Genotoxic effects of Streptozotocin. In: Gauthier EL, editor. *Streptozotocin: Uses, Mechanism of Action and Side Effects*. 1st ed. New York: Nova Science Publishers Inc.; 2014. p. 99-120.

- [55] Bolzán AD, Bianchi MS: Detection of incomplete chromosome elements and interstitial fragments induced by bleomycin in hamster cells using a telomeric PNA probe. *Mutat. Res.* 2004;554(1-2):1-8. DOI: 10.1016/j.mrfmmm.2004.02.016
- [56] Bolzán AD, Bianchi MS: Analysis of streptonigrin-induced incomplete chromosome elements and interstitial fragments in Chinese hamster cells using a telomeric PNA probe. *Environ. Mol. Mutagen.* 2004;44(4):277-282. DOI: 10.1002/em.20051
- [57] Bolzán AD, Bianchi MS: Analysis of streptozotocin-induced incomplete chromosome elements and excess acentric fragments in Chinese hamster cells using a telomeric PNA probe. *Mutat. Res.* 2005;570(2):237-244. DOI: 10.1016/j.mrfmmm.2004.11.008
- [58] Díaz-Flaqué MC, Bianchi MS, Bolzán AD: A comparative analysis of bleomycin-induced incomplete chromosome elements in two mammalian cell lines using a telomeric PNA probe. *Environ. Mol. Mutagen.* 2006;47(9):674-681. DOI: 10.1002/em.20254
- [59] Benkhaled L, Xunclá M, Caballín MR, Barrios L, Barquinero JF: Induction of complete and incomplete chromosome aberrations by bleomycin in human lymphocytes. *Mutat. Res.* 2008;637(1-2):134-141. DOI: 10.1016/j.mrfmmm.2007.07.013
- [60] Paviolo NS, Quiroga IY, Castrogiovanni DC, Bianchi MS, Bolzán AD: Telomere instability is present in the progeny of mammalian cells exposed to bleomycin. *Mutat. Res.* 2012;734(1-2):5-11. DOI: 10.1016/j.mrfmmm.2012.04.008
- [61] Paviolo NS, Castrogiovanni DC, Bolzán AD: The radiomimetic compound streptonigrin induces persistent telomere dysfunction in mammalian cells. *Mutat. Res.* 2014;760:16-23. DOI: 10.1016/j.mrfmmm.2013.11.009
- [62] Paviolo NS, Santiñaque FF, Castrogiovanni DC, Folle GA, Bolzán AD: The methylating agent streptozotocin induces persistent telomere dysfunction in mammalian cells. *Mutat. Res.* 2015;794:17-24. DOI: 10.1016/j.mrgentox.2015.09.007
- [63] Nguyen TV, Murray VA: Human telomeric DNA sequences are a major target for the antitumour drug bleomycin. *J. Biol. Inorg. Chem.* 2012;17(1):1-9. DOI: 10.1007/s00775-011-0818-3.
- [64] Nguyen HT, Murray V: The DNA sequence specificity of bleomycin cleavage in telomeric sequences in human cells. *J. Biol. Inorg. Chem.* 2012;17(8):1209-1215. DOI: 10.1007/s00775-012-0934-8.
- [65] Arutyunyan R, Gebhart E, Hovhannisyan G, Greulich KO, Rapp A: Comet-FISH using peptide nucleic acid probes detects telomeric repeats in DNA damaged by bleomycin and mitomycin C proportional to general DNA damage. *Mutagenesis* 2004;19(5):403-408. DOI: 10.1093/mutage/geh049
- [66] Arutyunyan R, Rapp A, Greulich KO, Hovhannisyan G, Haroutiunian S, Gebhart E: Fragility of telomeres after bleomycin and cisplatin combined treatment measured in human leukocytes with the Comet-FISH technique. *Exp Oncol.* 2005;27(1):38-42.

- [67] Hovhannisyan G, Rapp A, Arutyunyan R, Greulich KO, Gebhart E: Comet-assay in combination with PNA-FISH detects mutagen-induced DNA damage and specific repeat sequences in the damaged DNA of transformed cells. *Int. J. Mol. Med.* 2005;15(3):437-442 DOI: 10.3892/ijmm.15.3.437
- [68] Liu M, Hales BF, Robaire B: Effects of four chemotherapeutic agents, bleomycin, etoposide, cisplatin, and cyclophosphamide, on DNA damage and telomeres in a mouse spermatogonial cell line. *Biol. Reprod.* 2014;90(4):1-10. DOI: 10.1095/biolreprod.114.117754
- [69] Mekhail TM, Markman M: Paclitaxel in cancer therapy. *Expert. Opin. Pharmacother.* 2002;3(6):755-766. DOI: 10.1517/14656566.3.6.755
- [70] Schiff PB, Fant J, Horwitz SB: Promotion of microtubule assembly in vitro by taxol. *Nature* 1979;277(5698):665-667.
- [71] Schiff PB, Horwitz SB: Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA* 1980;77(3):1561-1565.
- [72] Multani AS, Li C, Ozen M, Yadav M, Yu DF, Wallace S et al.: Paclitaxel and water-soluble poly (L-glutamic acid)-paclitaxel, induce direct chromosomal abnormalities and cell death in a murine metastatic melanoma cell line. *Anticancer Res.* 1997;17(6D):4269-4274.
- [73] Multani AS, Li C, Ozen M, Imam AS, Wallace S, Pathak S: Cell-killing by paclitaxel in a metastatic murine melanoma cell line is mediated by extensive telomere erosion with no decrease in telomerase activity. *Oncol. Rep.* 1999;6(1):39-44. DOI: 10.3892/or.6.1.39
- [74] Multani AS, Chandra J, Mcconker J, Sen S, Cabral TF, Pathak S: Cell death in paclitaxel-dependent chinese hamster ovary cells is initiated by the loss of telomeric DNA repeats. *Oncol. Res.* 1999;11(10):455-460. DOI:
- [75] Mo Y, Gan Y, Song S, Johnston J, Xiao X, Wientjes MG et al.: Simultaneous targeting of telomeres and telomerase as a cancer therapeutic approach. *Cancer Res.* 2003;63(3):579-585.
- [76] Johnston JS, Johnson A, Gan Y, Wientjes MG, Au JL: Synergy between 3'-azido-3'-deoxythymidine and paclitaxel in human pharynx FaDu cells. *Pharm. Res.* 2003 ;20(7):957-961.
- [77] Park JE, Woo SR, Kang CM, Juhn KM, Ju YJ, Shin HJ et al.: Paclitaxel stimulates chromosomal fusion and instability in cells with dysfunctional telomeres: implication in multinucleation and chemosensitization. *Biochem. Biophys. Res. Commun.* 2011;404(2):615-621. DOI: 10.1016/j.bbrc.2010.12.018
- [78] Ishibashi T, Lippard SJ: Telomere loss in cells treated with cisplatin. *Proc. Natl. Acad. Sci. U.S.A.* 1998;95(8):4219-4223.

- [79] Paul M, Murray V: Use of an automated capillary DNA sequencer to investigate the interaction of cisplatin with telomeric DNA sequences. *Biomed. Chromatogr.* 2012;26(3):350-354. DOI: 10.1002/bmc.1664
- [80] Murray V, Kandasamy N: The sequence specificity of the anti-tumour drug, cisplatin, in telomeric DNA sequences compared with consecutive guanine DNA sequences. *Anticancer Agents Med. Chem.* 2012;12(3):177-181. DOI: 10.2174/187152012800228742
- [81] Nguyen HT, Galea AM, Murray V. The interaction of cisplatin with a human telomeric DNA sequence containing seventeen tandem repeats. *Bioorg. Med. Chem. Lett.* 2013;23(4):1041-1045. DOI: 10.1016/j.bmcl.2012.12.021
- [82] Burger AM, Double JA, Newell DR: Inhibition of telomerase activity by cisplatin in human testicular cancer cells. *Eur. J. Cancer.* 1997;33(4):638-644. DOI: 10.1016/S0959-8049(96)00521-7
- [83] Błasiak J, Kadłubek M, Kowalik J, Romanowicz-Makowska H, Pertyński T: Inhibition of telomerase activity in endometrial cancer cells by selenium-cisplatin conjugate despite suppression of its DNA-damaging activity by sodium ascorbate. *Teratog. Carcinog. Mutagen.* 2002;22(1):73-82.
- [84] Manet I, Manoli F, Zambelli B, Andreano G, Masi A, Cellai L et al.: Affinity of the anthracycline antitumor drugs Doxorubicin and Sabarubicin for human telomeric G-quadruplex structures. *Phys. Chem. Chem. Phys.* 2011;13(2):540-551. DOI: 10.1039/c0cp00898b
- [85] Zhang RG, Guo LX, Wang XW, Xie H: Telomerase inhibition and telomere loss in BEL-7404 human hepatoma cells treated with doxorubicin. *World J. Gastroenterol.* 2002;8(5):827-831. DOI: 10.3748/wjg.v8.i5.827
- [86] Kiyozuka Y, Yamamoto D, Yang J, Uemura Y, Senzaki H, Adachi S et al.: Correlation of chemosensitivity to anticancer drugs and telomere length, telomerase activity and telomerase RNA expression in human ovarian cancer cells. *Anticancer Res.* 2000;20(1A):203-212.
- [87] Elmore LW, Rehder CW, Di X, McChesney PA, Jackson-Cook CK, Gewirtz DA et al.: Adriamycin-induced senescence in breast tumor cells involves functional p53 and telomere dysfunction. *J. Biol. Chem.* 2002;277(38):35509-35515. DOI: 10.1074/jbc.M205477200
- [88] Park KH, Rha SY, Kim CH, Kim TS, Yoo NC, Kim JH et al.: Telomerase activity and telomere lengths in various cell lines: changes of telomerase activity can be another method for chemosensitivity evaluation. *Int. J. Oncol.* 1998;13(3):489-495. DOI: 10.3892/ijo.13.3.489
- [89] Spallarossa P, Altieri P, Aloï C, Garibaldi S, Barisione C, Ghigliotti G et al.: Doxorubicin induces senescence or apoptosis in rat neonatal cardiomyocytes by regulating the

- expression levels of the telomere binding factors 1 and 2. *Am. J. Physiol. Heart Circ. Physiol.* 2009;297(6):H2169-2181. DOI: 10.1152/ajpheart.00068
- [90] Buttiglieri S, Ruella M, Risso A, Spatola T, Silengo L, Avvedimento EV et al.: The aging effect of chemotherapy on cultured human mesenchymal stem cells. *Exp. Hematol.* 2011;39(12):1171-1181. DOI: 10.1016/j.exphem.2011.08.009
- [91] Li P, Hou M, Lou F, Björkholm M, Xu D: Telomere dysfunction induced by chemotherapeutic agents and radiation in normal human cells. *Int. J. Biochem. Cell Biol.* 2012;44(9):1531-1540. DOI: 10.1016/j.biocel.2012.06.020
- [92] Liu M, Maselli J, Hales BF, Robaire B: The effects of chemotherapy with bleomycin, etoposide, and cis-platinum on telomeres in rat male germ cells. *Andrology* 2015;3(6):1104-1112. DOI: 10.1111/andr.12102
- [93] Jeyapalan J, Leake A, Ahmed S, Saretzki G, Tilby M, von Zglinicki T: The role of telomeres in Etoposide induced tumor cell death. *Cell Cycle* 2004;3(9):1169-1176.
- [94] Christman JK: 5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* 2002;21(35):5483-5495. DOI: 10.1038/sj.onc.1205699
- [95] Motevalli A, Yasaei H, Virmouni SA, Slijepcevic P, Roberts T: The effect of chemotherapeutic agents on telomere length maintenance in breast cancer cell lines. *Breast Cancer Res. Treat.* 2014;145(3):581-591. DOI: 10.1007/s10549-014-2975-x
- [96] Zhang X, Li B, de Jonge N, Björkholm M, Xu D: The DNA methylation inhibitor induces telomere dysfunction and apoptosis of leukemia cells that is attenuated by telomerase over-expression. *Oncotarget* 2015;6(7):4888-4900. DOI: 10.18632/oncotarget.2917
- [97] Choudhury SR, Cui Y, Milton JR, Li J, Irudayaraj J: Selective increase in subtelomeric DNA methylation: an epigenetic biomarker for malignant glioma. *Clin. Epigenetics.* 2015;7:107. DOI: 10.1186/s13148-015-0140-y.
- [98] Grindey GB, Hertel LW, Plunkett W: Cytotoxicity and antitumor activity of 2', 2'-difluorodeoxycytidine (Gemcitabine). *Cancer Invest.* 1990;8(2):313.
- [99] Su CH, Chu WC, Lan KH, Li CP, Chao Y, Lin HC et al.: Gemcitabine causes telomere attrition by stabilizing TRF2. *Eur. J. Cancer.* 2012;48(18):3465-3474. DOI: 10.1016/j.ejca.2012.04.015
- [100] Hu JL, Xue YC, Xie MY, Zhang R, Otani T, Minami Y et al.: A new macromolecular antitumor antibiotic, C-1027. I. Discovery, taxonomy of producing organism, fermentation and biological activity. *J. Antibiot. (Tokyo)* 1988;41:1575-1579.
- [101] Otani T, Minami Y, Marunaka T, Zhang R, Xie MY: A new macromolecular antitumor antibiotic, C-1027. II. Isolation and physico-chemical properties. *J. Antibiot. (Tokyo)* 1988;41:1580-1585.

- [102] Zhen YS, Ming XY, Yu B, Otani T, Saito H, Yamada Y. A new macromolecular antitumor antibiotic, C-1027. III. Antitumor activity. *J. Antibiot. (Tokyo)* 1989;42:1294-1298.
- [103] Chen Y, Yu D, Zhang C, Shang B, He H, Chen J et al.: Lidamycin inhibits tumor initiating cells of hepatocellular carcinoma Huh7 through GSK3 β / β -catenin pathway. *Mol. Carcinog.* 2015;54(1):1-8. DOI: 10.1002/mc.22069
- [104] McHugh MM, Gawron LS, Matsui S, Beerman TA: The antitumor enediyne C-1027 alters cell cycle progression and induces chromosomal aberrations and telomere dysfunction. *Cancer Res.* 2005;65(12):5344-5351. DOI: 10.1158/0008-5472.CAN-05-0015
- [105] Downes CS, Clarke DJ, Mullinger AM, Giménez-Abián JF, Creighton AM, Johnson RT: A topoisomerase II-dependent G2 cycle checkpoint in mammalian cells. *Nature* 1994;372(6505):467-470. DOI: 10.1038/372467a0
- [106] Chen L, Zhu X, Zou Y, Xing J, Gilson E, Lu Y et al.: The topoisomerase II catalytic inhibitor ICRF-193 preferentially targets telomeres that are capped by TRF2. *Am. J. Physiol. Cell. Physiol.* 2015;308(5):C372-377. DOI: 10.1152/ajpcell.00321.2014
- [107] Hsieh MH, Tsai CH, Lin CC, Li TK, Hung TW, Chang LT et al.: Topoisomerase II inhibition suppresses the proliferation of telomerase-negative cancers. *Cell. Mol. Life Sci.* 2015;72(9):1825-1837. DOI: 10.1007/s00018-014-1783-0
- [108] Dronkert ML, Kanaar R: Repair of DNA interstrand cross-links. *Mutat. Res.* 2001;486(4):217-247. DOI:10.1016/S0921-8777(01)00092-1
- [109] Sgura A, De Amicis A, Stronati L, Cinelli S, Pacchierotti F, Tanzarella C: Chromosome aberrations and telomere length modulation in bone marrow and spleen cells of melphalan-treated p53 $^{+/-}$ mice. *Environ. Mol. Mutagen.* 2008;49(6):467-475. DOI: 10.1002/em.20405
- [110] Malet-Martino M, Martino R: Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): a review. *Oncologist* 2002;7(4):288-323. DOI: 10.1634/theoncologist.7-4-288
- [111] Kuranaga N, Shinomiya N, Mochizuki H: Long-term cultivation of colorectal carcinoma cells with anti-cancer drugs induces drug resistance and telomere elongation: an in vitro study. *BMC Cancer* 2001;1:10. DOI: 10.1186/1471-2407-1-10
- [112] Lin G, Lin MC, Lin S, Yao H, Yu S, Yi W et al.: Early growth response protein-1 promoter-mediated synergistic antitumor effect of hTERTC27 gene therapy and 5-Fluorouracil on nasopharyngeal carcinoma. *Cancer Biother. Radiopharm.* 2012;27(7):434-441. DOI: 10.1089/cbr.2011.1153
- [113] Lin G, Chen Q, Yu S, Lin S, Yao H, Ding Z et al.: Overexpression of human telomerase reverse transcriptase C-terminal polypeptide sensitizes HeLa cells to 5-fluorouracil-induced growth inhibition and apoptosis. *Mol. Med. Rep.* 2014;9(1):279-284. DOI: 10.3892/mmr.2013.1777

