1 Targeting cancer through the epigenetic features of

2 telomeric regions

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7 María Isabel Vaquero-Sedas¹ and Miguel Ángel Vega-Palas^{1,*}

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9 ¹Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla,
10 Seville, Spain.

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15 *Correspondence: vega-palas@ibvf.csic.es (M.A. Vega-Palas)

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Abstract

The integrity of the chromatin associated with telomeric regions, which include telomeres and subtelomeres, is essential for telomeres function and cell viability. Whereas human subtelomeres are heterochromatic, telomeres are labeled with euchromatic marks like H4K20me1 and H3K27ac in most commonly studied human cell lines. The epigenetic marks of human telomeric regions influence oncogenic processes. Indeed, different drugs that decrease their genome-wide levels are currently being used or tested in specific cancer therapies. These drugs can challenge cancer by altering the function of key cellular proteins. However, they should also compromise oncogenic processes by modifying the epigenetic landscape of telomeric regions. We believe that studies of telomeric chromatin structure and telomeres dysfunction should help to design epigenetic therapies for cancer treatment.

Telomeres are required for sustained cell proliferation

Telomeres protect the end of chromosomes, facilitate their replication and are required for sustained cell proliferation during developmental processes and neoplastic transformation [1, 2]. Telomeric DNA is composed of short G-rich tandem telomeric repeats, which in mammals have the sequence TTAGGG. Most human somatic cells, shorten progressively their telomeres through cell divisions due to the inability of the replication machinery to copy the 3' end of linear chromosomes. This end-replication problem, together with terminal processing activities, can lead to a critical shortening of telomeres that impair their function and, as a consequence, cell growth. However, certain types of human cells, like stem mother cells, can replicate their telomeres thanks to the action of the enzyme telomerase, thus allowing their continuous growth and regenerative capacity [1, 2]. Therefore, telomerase plays a pivotal role in cell biology [3-5].

In order to sustain continuous cell proliferation cancer cells need to maintain their telomeres either by the action of telomerase or by an alternative recombinational mechanism known as ALT (Alternative Lengthening of Telomeres) [1, 2]. Therefore, interfering with any of these two maintenance mechanisms can compromise cancer cells growth and oncogenic processes.

Human telomeric regions have a bimodal chromatin organization

Like the length of telomeres, the chromatin organization of telomeric regions influences telomeres function and cell proliferation [6]. Two main kinds of chromatin are found within the nucleus of eukaryotic cells: euchromatin and heterochromatin. Whereas euchromatin has an open chromatin conformation

that can allow transcription, heterochromatin is compacted and generally silenced [7, 8].

Heterochromatin can be constitutive or facultative. Constitutive heterochromatin can be observed in interphase nuclei as densely-stained nuclear areas known as chromocenters. These chromocenters contain pericentromeric repetitive elements, including transposons, and associate with repressive epigenetic marks such as DNA methylation, H3K9me3 and histones hypoacetylation. The concerted interactive action of these marks cooperate to maintain the integrity of heterochromatin and control mobile elements, which guaranty genome stability [7, 8]. When euchromatin acquire a close conformation, it can be referred to as facultative heterochromatin. This kind of chromatin regulates development and differentiation by silencing specific genes, thus leading to the onset of specific gene expression programs. Facultative heterochromatin is characterized by the absence of DNA methylation and the presence of H2AK119ub and/or H3K27me3, which are established by the polycomb repressive complexes 1 and 2, respectively [9, 10]. Therefore, although both types of heterochromatin, constitutive and facultative, can inhibit transcription, they rely on different molecular mechanisms. Since facultative heterochromatin is not the subject of this manuscript, hereinafter we will refer to constitutive heterochromatin as heterochromatin.

The chromatin organization of human telomeric regions is bimodal. Human subtelomeres contain repetitive elements, are heterochromatic and exhibit dense and widespread H3K9me3, DNA methylation and histones hypoacetylation. In turn, although telomeres are repetitive sequences, they do not have enriched levels of heterochromatic marks. Instead, they are labeled with

H4K20me1 and H3K27ac in most commonly studied human cell lines [11-13]. In addition, human telomeres associate with shelterin proteins, which are essential for telomeres function. The central role that shelterins play in telomeres biology has been recently reviewed [1].

Following, we describe the epigenetic features of human telomeric regions and their relationship with different types of cancer. In addition, we highlight that different anti-cancer drugs that target these epigenetic features or their writers impair telomeres function. Consequently, we reason that the output of such drugs on cancer progression could be due, in part, to telomeres dysfunction. We propose that these epigenetic drugs should be tested for efficiency in challenging the function of telomeres and the proliferative capacity of a wide variety of cancer cells. These studies could help to design precise epigenetic therapies for specific types of cancer.

Two relevant euchromatic marks label human telomeres

Human telomeres are labelled with H4K20me1 and H3K27ac in most commonly studied human cell lines including embryonic stem cells, primary cells and certain cancer cells [13]. The methylation of H4K20 at telomeres should be achieved by SET8, which is the only known *in vivo* human H4K20 mono-methyltransferase. In turn, although H3K27 can be acetylated by p300 and CBP, telomeres have been proposed to acquire the H3K27ac mark through the action of p300. Unlike CBP, p300 associates with human telomeres and acetylates the shelterin protein TRF2, which is required for proper telomeres function (Figure 1A) [13-18].

H4K20me1 and H3K27ac play important roles in different aspects of the DNA metabolism and in cell proliferation. H4K20me1 is enriched at the coding

region of active genes and associates with chromatin compaction during mitosis. Although this epigenetic mark is related to transcriptional activation it has also been associated with repression [15, 16, 19]. H4K20me1 and the methyltransferase activity of SET8 are involved in essential cellular processes including DNA replication, mitosis and DNA repair. They control these processes by modulating gene expression and through methylation of key regulatory proteins like PCNA or p53, which can promote tumorigenesis [14-16].

H3K27ac is usually found at active promoters and enhancers and is associated with gene expression. As mentioned above, H3K27ac can be established by p300 and CBP [18]. These proteins are transcriptional co-activators that connect specific transcription factors with the general transcriptional machinery and stimulate the expression of a wide variety of genes. p300/CBP regulate important cellular processes including the DNA damage response, cell cycle progression, cell growth and differentiation. They regulate these processes by controlling gene expression and also by acetylating key cellular proteins like some components of the transcriptional and replication machinery [18]. Hence, p300/CBP and SET8 influence cell proliferation by regulating the expression and/or activity of relevant cellular proteins.

Human subtelomeres are heterochromatic and control telomeres function

Subtelomeric regions in humans exhibit heterochromatic features like H3K9me3, DNA methylation and histones hypoacetylation [20-22]. Different sources of evidence argue that subtelomeric heterochromatin plays a major role in the biology of human telomeres. First, mutations in different proteins involved in the

formation of heterochromatin impair subtelomeric heterochromatin formation and can eventually lead to telomere shortening, to a p53-dependent DNA damage response, to the appearance of TIFs and to cell death. Among these proteins are a histone H3K9 trimethyltransferase (SUV39H1), a structural protein that binds to H3K9me3 (HP1 α), a DNA methyltransferase (DNMT3B), a histone deacetylase (SIRT6) and a DNA helicase required for heterochromatin assembly, which mutation causes the age-related Syndrome of Werner (WRN) [22-28]. In addition, increasing the levels of the DNA methyltransferase DNMT3A or HP1 α at telomeric regions by fusing them to the shelterin protein TRF1 can alter the function of telomeres [29, 30]. Besides, human subtelomeric heterochromatin can silence the expression of nearby located genes and of telomeric repeats containing RNAs (TERRA), which are essential for telomeres function [31-36].

Human TERRA molecules are transcribed from subtelomeric regions towards telomeres and arise mainly from the 20q and Xq subtelomeres. These TERRA transcripts contain subtelomeric and telomeric sequences and associate with TRF2, HP1 and H3K9me3. TERRA expression is controlled by subtelomeric CpG islands that undergo DNA methylation and are near to CTCF binding sites. Depletion of CTCF reduces cohesin and RNA polymerase II binding to subtelomeres and leads to a decrease in TERRA transcription. In addition, depletion of either CTCF or cohesin subunit Rad21 causes telomeres dysfunction. Hence, nuclear organization proteins like CTCF and cohesin components associate with subtelomeres and control TERRA expression, which has to be properly modulated. Decreasing TERRA levels through siRNA knock-down or deletion of subtelomeric sequences in 20q induce a telomeric DNA damage response that leads to TIF formation. In turn, increased levels of TERRA

caused by the loss of DNA methylation in ICF syndrome cells lead to the formation of R-loops at telomeres and subtelomeres and cause telomeres dysfunction [31-34, 36-39]. Therefore, the control of TERRA by subtelomeric heterochromatin seems to be important for telomeres function.

Additional evidence also supports a relevant role for subtelomeric heterochromatin in telomeres biology. Super-resolution microscopy has revealed that telomeres form compact and protective globular structures through a complex network of interactions between shelterin subunits and telomeric DNA [40]. Mutations that abrogate shelterin proteins cause an increase in telomere volume and accessibility that induces a p53-dependent DNA damage response at telomeres [1, 12, 40]. In contrast, impairment of heterochromatin formation through treatments that inhibit histone deacetylation, DNA methylation or H3K9me3 does not disrupt the telomeric compact structures [40]. However, the loss of heterochromatin can also lead to a p53-dependent telomeric DNA damage response [22-28]. These results do not support a protective role for heterochromatin at true telomeres. Whereas the uncapping of telomeres due to mutations or release of shelterin should damage true telomeres, the telomeric DNA damage generated by heterochromatin impairment might occur at subtelomeres. This notion is supported by the fact that p53 binds to p53 non-canonical binding sites at subtelomeric regions under DNA damage conditions, stimulate TERRA transcription, prevent TIFs formation and protect telomeres from DNA degradation [41].

Subtelomeric DNA damage might contribute to the onset of cellular senescence. Cellular senescence has been proposed to arise from a telomeric DNA damage signal that involves the accumulation of 5 TIFs per cell after

successive cell divisions. These TIFs are thought to originate from the progressive shortening of telomeres. However, although the number of TIFs in aging cells correlates with bulk telomere shortening, TIFs in individual cells do not necessarily localize to the shortest telomeres [33, 42, 43]. The appearance of TIFs in long telomeres could be explained by alterations of subtelomeric heterochromatin. A global loss of heterochromatin has been reported in aging mutants and old cells. This age-related heterochromatin decrease, which also occurs at subtelomeric regions, has been proposed as a potential determinant of cellular and human aging. Therefore, subtelomeric heterochromatin disruption together with telomere shortening might contribute to generate the dysfunctional telomeres that activate the p53 senescence pathway [22, 33, 44][12]. Notably, both, telomeres and subtelomeres, have been found to associate with the DNA damage protein 53BP1 in aging cells [12].

Taking together all the aforementioned results and considering that telomeres do not show enrichment of heterochromatic marks in most commonly studied human cell lines [13], we conclude that subtelomeric heterochromatin plays a relevant role in the biology of human telomeres (Figure 1A). A similar role of subtelomeric heterochromatin can be envisioned for organisms like *Arabidopsis thaliana* [45, 46], yeast [47-50] or *Drosophila melanogaster* [51, 52].

The epigenetic marks present in human telomeric regions are targeted by anti-cancer drugs

Epigenetic marks control repetitive elements and set specific gene expression programs during development and differentiation. Not surprisingly, these expression programs are altered in multiple cancer cells due to aberrant

epigenome reprogramming events. These reprogramming events can originate from alterations in different writers, readers and erasers of the epigenetic information. Therefore, the identification of drugs that target these proteins has become a forefront task [53].

The genome-wide distributions of H4K20me1, H3K27ac and heterochromatic marks play important roles in different aspects of the DNA metabolism and in cell proliferation. Since these epigenetic marks have been associated with different kinds of tumours, their writers are being considered as targets of cancer therapies. [8, 14, 18, 53]. SET8 is overexpressed in several types of cancers and current efforts are focusing in finding out drugs that specifically inhibit this protein with the aim of controlling undesired cell proliferation [14-16]. Similarly, mutations or miss-expression of p300/CBP and alterations of H3K27ac associate with certain cancer types. Indeed, inhibitors of p300/CBP are being assayed in preclinical studies [18, 54, 55]. Two of these inhibitors, C646 and L002, can impair the growth of human melanoma, prostate and breast cancer cells [56-58].

Several drugs that decrease the levels of heterochromatic marks like DNA methylation or histones hypoacetylation have already been approved for specific cancer treatments [53, 59, 60]. These epigenetic drugs include nucleoside analogs like azacitidine or decitabine, which impair DNA methylation, and histone deacetylase inhibitors (HDACi) like vorinostat, romidepsin, belinostat and panobinostat. Whereas the DNA methylation inhibitors are being used for the treatment of myelodysplastic syndromes, the histone deacetylase inhibitors have been approved for the treatment of peripheral/cutaneous T cell lymphomas and multiple myelomas. However, both kinds of drugs possess activity against other

tumor malignancies and are currently being tested for the treatment of different cancer types [53, 59, 60].

Nucleoside analogs incorporate to DNA during replication and bind covalently to DNA methyltransferases causing their degradation and the passive demethylation of DNA through cell divisions. In addition, they can lead to a DNA damage response and cytotoxicity in a concentration dependent manner [61]. Nucleoside analogs have different outputs on gene expression depending on the genomic loci that they target. Whereas repetitive elements and certain genes can be de-repressed, other genes might become silenced. Despite being a hallmark of heterochromatin and repetitive elements silencing, mammals DNA methylation can influence gene expression in two different ways. On the one hand, DNA methylation can repress transcription when targeted to CpG islands and regulatory regions, where it can be found together with H3K9me2. On the other hand, DNA methylation can be established in the body of genes and potentiate transcription [62-64]. The aberrant acquisition of these two types of gene methylation can lead to neoplastic transformation either by silencing of tumor suppressor genes or through the activation of oncogenic pathways. In both cases, restoring normal methylation patterns by using nucleoside analogs can lead to antitumor activity [63, 65]. Interestingly, some types of cancer, such as myelodysplastic syndromes, frequently associate with mutations in the DNA methyltransferase DNMT3A or the DNA demethylase TET2 [66].

As for the DNA methylation inhibitors, the anti-cancer activity of HDACi has been related, in part, with the reactivation of tumor suppressor genes that are hypomethylated. However, HDACi can also influence the activity of acetylated non-histone proteins, including transcription factors like p53 [67]. The balance of

histones acetylation mediated by acetyl transferases and deacetylases regulates chromatin compaction and gene expression. The addition of acetyl groups to histones lysines neutralize their positive charge and loosens chromatin interactions leading to an open conformation that can allow transcription. In turn, histones deacetylation restores lysines positive charges and leads to a closed conformation and silencing. Consequently, HDACi can cause heterochromatin disaggregation and increase expression of mobile elements as well as specific genes [8, 68-70]. [67].

Epigenetic drugs can impair human telomeres function

In general, the aforementioned drugs can compromise cancer cells proliferation by modulating the expression and/or post-translational modifications of key cellular proteins including oncoproteins, tumour-suppressor proteins, proteins related with the immune response and others. However, these drugs could also interfere with the functions of telomeres by modifying their epigenetic landscape, which should contribute to compromise cancer cells viability (Figure 1B). This notion is supported by previously published results (Table 1). Inhibition of p300/CBP by C646 leads to decreased levels of histones acetylation, telomere shortening and repression of subtelomeric genes [56-58, 71]. Thus, C646 might influence telomeres function at telomeric and subtelomeric levels. It might impair the acetylation of H3K27 and TRF2 at telomeres and unbalance the homeostasis of subtelomeric acetylation [13, 17, 71]. In addition, Trichostatin A, a histone deacetylase inhibitor that impairs the growth of different cancer cells [72-75], has been shown to release TPE and increase telomere sister chromatid exchange (T-SCE) [32, 76, 77]. Besides, two DNA methylation drugs that have been approved

for the treatment of leukaemia can also induce telomere dysfunction. Whereas decitabine shortens telomeres and induces senescence, azacitidine decreases subtelomeric DNA methylation, releases TPE, induces TIFs, increases T-SCE and triggers apoptosis [78-81]. We speculate that the telomere damage induced by the drugs that target heterochromatin might be related TERRA. Ectopic expression of TERRA caused by the loss of subtelomeric silencing might lead to genome instability, as previously reported for ICF syndrome cells with low levels of subtelomeric DNA methylation [37].

The epigenetic features of human telomeric regions as targets of cancer therapies

Since drugs targeting the epigenetic features of human telomeric regions can impair telomeres function, telomeres should be monitored when cancer therapies based on the use of such drugs are applied. It would be interesting to determine how important are the impacts of epigenetic drugs on telomeres dysfunction when compared to their impacts on key cellular proteins in terms of controlling cell proliferation. These impacts should be evaluated in cancer cells and in normal cells in order to assess the side-effects that are inherent to multiple cancer therapies.

Different cancer cell lines could have distinct marks or different levels of epigenetic marks at telomeric regions. Hence, they could respond differently to epigenetic drugs. Monitoring the epigenetic features of telomeric regions and the influence of epigenetic drugs on telomeres function could help to design specific epigenetic therapies for particular cancer types. In addition, these kinds of studies

should also help to minimize the side-effects of epigenetic drugs due to their impacts on normal cells telomeres.

As previously mentioned, cancer cells can maintain their telomeres through the action of the enzyme telomerase or by an alternative recombinational mechanism known as ALT [1, 2]. Unlike different types of telomerase-positive cancer cells, an osteosarcoma cell line that maintains telomeres through ALT (U2OS) has enriched levels of H3K9me3 at telomeres [13]. Similarly, a neuroblastoma cell line that also undergoes ALT (LAN6) [82, 83] has enriched levels of H3K9me3 at telomeres too (our unpublished results). In addition, U2OS cells and other types of ALT cancer cells have low levels of subtelomeric DNA methylation in specific subtelomeric regions [13, 20]. Considering that about 15% of the human cancers undergo ALT, it will be interesting to ascertain whether high levels of telomeric H3K9me3 and low levels of subtelomeric DNA methylation are hallmarks of ALT cells.

Since U2OS cells and certain telomerase-positive cancer cells have different levels of heterochromatic marks at telomeres and subtelomeres, they could respond differently to epigenetic drugs targeting heterochromatin. Interestingly, trabectedin, an anti-tumour drug which mechanism of action is not yet fully understood, has been proposed to differentially alter the viability of telomerase-positive and ALT-positive cancer cells [84]. It will be interesting to ascertain whether this differential activity of trabectedin rely on the chromatin organization of telomeric regions.

Concluding remarks

Telomeric regions in humans have a bimodal chromatin organization. Whereas human subtelomeres are enriched in heterochromatic marks, telomeres are labelled with euchromatic epigenetic modifications like H4K20me1 and H3K27ac. The methylation of H4K20 at telomeres should be achieved by SET8, which is the only known *in vivo* human H4K20 mono-methyltransferase. In turn, H3K27 has been proposed to acquire the H3K27ac mark through the action of p300.

Epigenetic drugs targeting heterochromatin, H4K20me1, H3K27ac or their writers are currently being used or tested in specific cancer therapies. These drugs can challenge cell proliferation by altering the expression and/or post-translational modifications of key cellular proteins. However, they can also compromise cell proliferation by modifying the epigenetic landscape of telomeric regions. Hence, telomeres should be considered when cancer therapies based on the use of such drugs are applied. Monitoring the epigenetic features of telomeric regions and the influence of epigenetic drugs on telomeres function could help to design epigenetic therapies. Since epigenetic drugs could cooperatively challenge telomeres function, they could be combined and tested for efficiency in generating telomeres dysfunction of a wide variety of tumour cells. Their anti-telomere activity could efficiently contribute to challenge the progression of multiple cancer types.

Clinical trials using different combinations of epigenetic drugs are currently being performed. In addition, trials combining epigenetic drugs with Immune Checkpoint Blockade (ICB) drugs are also underway [60]. ICB drugs potentiate the action of the immune system against cancer cells by blocking the inhibitory receptors present on T cells, thus inducing their activation (reference). The effectiveness of ICB therapy can be stimulated by drugs targeting

heterochromatin. On the one hand, heterochromatin drugs can potentiate the action of T cells by releasing silencing of particular genes that are important for the immune response. These genes might become silenced during the so-called T cell exhaustion process, which results from prolonged antigen stimulation and has been observed during viral infection and cancer. Since one of the characteristics of T cell exhaustion is increased expression of inhibitory receptor, ICB drugs also contribute to revert this T cell dysfunction [60, 85, 86]. On the other hand, in cancer cells, heterochromatin drugs de-repress the expression of long terminal repeat retrotransposons, also known as endogenous retroviruses (ERVs), which leads to an increase of double-strand RNA molecules and the activation of defense dsRNA recognition pathways. These signalling pathways stimulate the expression of immune response genes leading to a state that mimics viral infection, inhibits cells growth and sensitizes against the action of ICB drugs. Hence, the disruption of heterochromatin at ERVs seems to play a relevant role in the response to epigenetic therapy. Notably, the immune response caused by DNA methylation inhibitors in some cellular contexts can account for a high proportion of its inhibitory effect on growth [60, 87, 88].

The aforementioned data reveal that epigenetic drugs can impair cancer progression at different molecular levels. In this context, we aim to highlight that the generation of telomeres dysfunction adds a new level of complexity to the action of epigenetic drugs. Hence, comprehensive studies of telomeric chromatin structure and function should help to define the rationale for combining epigenetic drugs among them and with other oncogenic drugs like those that induce ICB (see Outstanding Questions).

Glossary

ALT: Alternative Lengthening of Telomeres. Recombinational mechanism that allows telomere maintenance and cancer progression in the absence of telomerase.

Cohesin: Protein complex that control sister chromatid cohesion and other cellular processes including the regulation of transcription and chromatin architecture.

CTCF: CCCTC-binding factor involved in different cellular processes including the regulation of recombination, transcription and chromatin architecture.

Epigenetic drugs: Drugs that modify the epigenetic status of cells at genome-wide level. Although only DNA methylation and histone deacetylase inhibitors have been approved to treat cancer, many others are under clinical trials.

ICB: Immune Checkpoint Blockade. Inhibition of regulators that prevent the immune system from attacking cells indiscriminately. ICB improves cancer treatment. Different ICB drugs like pembrolizumab, nivolumab, atezolizumab and ipilimumab have already been approved for the treatment of cancer. The 2018 Nobel Prize in Physiology and Medicine has acknowledged ICB therapy.

R-loop: Three-stranded nucleic acid structure in which RNA invades double stranded DNA generating a DNA:RNA hybrid and the associated single-stranded DNA. R-loops can cause DNA damage and genome instability.

Shelterin: Protein complex that binds to telomeres, protect them and facilitate their replication. It is composed of six proteins in humans (TRF1, TRF2, Rap1, TPP1, TIN2 and POT1).

TIF: Telomere Induced Foci. Telomeres associated with DNA damage response proteins as visualized by microscopy. TIF reflect telomeres dysfunction.

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Table 1. Effect of some epigenetic drugs on telomeres biology			
Drug	Action	Oncogenic activity	Influence on telomeres
Azacitidine	HMA	FDA-approved for acute myeloid leukaemia (AML) and chronic myelomonocytic leukaemia (CML). Clinical trials against recurrent head, neck and lung cancer undergoing [60]	Decreases subtelomeric DNA methylation and Increases T-SCE in Hut-78 cells (peripheral T-cell lymphoma) and SW480 cells (colon adenocarcinoma) [79]
			Shortens telomeres and induces TIFs and apoptosis in KG1A and HEL cells (AML) [80]
			Releases TPE in JM-1 ES cells [81]
Decitabine	HMA	FDA-approved for acute myeloid leukaemia (AML) [60]	Shortens telomeres and induces senescence in K-562, MEG-01 and KBM-5 cells (CML) [78]
Thricostatine A	HDACi	Active against pancreatic, renal, hepatic or brain tumour cells [72-75]	Releases TPE in HeLa cells (cervix epitheloid carcinoma) [32]
			Releases TPE in C33-A cells (cervix squamous cell carcinoma) [76]
			Increases T-SCE in WI38VA13 cells (lung fibroblast SV40 transformed) and GM847 cells (skin fibroblast SV40 transformed) [77]
C646	P300/CBPi	Active against melanoma, prostate or breast cancer cells [56-58]	Shortens telomeres and increases TPE in J1 ES cells [71]

Abbreviations: HMA, DNA hypomethylating agent; HDACi, histone deacetylase inhibitor; P300/CBPi, P300/CBP inhibitor; T-SCE, telomere sister chromatid exchange.

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Figure 1. The epigenetic landscape of human telomeric regions influence telomeres function and cell proliferation. (A) Epigenetic marks of telomeric regions in proliferative human cells. Most of the commonly studied human cell lines have heterochromatic subtelomeres and are labelled with H4K20me1 and H3K27ac at telomeres. Whereas H4K20me1 could be established by SET8, H3K27ac should be provided by p300, which associates with telomeres and also acetylates TRF2 [8, 13, 14, 17, 18]. (B) Influence of the epigenetic landscape of human telomeric regions on cell proliferation. Disruption of subtelomeric heterochromatin by mutation of the heterochromatin assembly machinery or treatment with inhibitors of DNA methylation or histones acetylation leads to dysfunctional telomeres and impaired cell proliferation [8, 77, 79, 80]. Similarly, disruption or inhibition of both, p300 and SET8, leads to impaired cell proliferation, which might be influenced by their contribution to the establishment of the telomeric marks (indicated by discontinuous arrows). In the case of p300, telomeres dysfunction contributes to impair cell proliferation due, at least in part, to the lack of TRF2 acetylation [13, 14, 17, 18, 71].

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