Implications of telomeres and telomerase in endometrial pathology

D.K. Hapangama1,2,*, A. Kamal1,3, and G. Saretzki4

1Department of Women’s and Children’s Health, Institute of Translational Medicine, University of Liverpool, Liverpool, L8 7SS, UK
2Liverpool Women’s Hospital NHS Foundation Trust, Crown Street, Liverpool L8 7SS, UK
3The National Center for Early Detection of Cancer, Oncology Teaching Hospital, Baghdad Medical City, Baghdad, Iraq
4Institute for Ageing and Institute for Cell and Molecular Biosciences, Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne, NE4 5PL, UK

*Correspondence address. E-mail: dharani@liv.ac.uk

Submitted on July 19, 2016; resubmitted on November 10, 2016; editorial decision on November 16, 2016; accepted on December 2, 2016

TABLE OF CONTENTS

- Introduction
- Method
- Telomeres
  - Structure
  - Telomere shortening
  - Functions of telomeres
  - Telomere maintenance
- Telomerase
  - Telomerase components
  - Functions of telomerase
- The endometrium
  - Telomerase and telomeres in endometrial tissue: functional relevance
  - Hormonal regulation of telomerase in epithelial cells
  - Endometrial stem cells and telomerase
  - Role of telomerase in pathological conditions of the endometrium
- Future directions and wider implications of interventions into telomerase biology
- Conclusion

BACKGROUND: Eukaryotic chromosomal ends are linear and are protected by nucleoprotein complexes known as telomeres. The complex structural anatomy and the diverse functions of telomeres as well as the unique reverse transcriptase enzyme, telomerase that maintains telomeres are under intensive scientific scrutiny. Both are involved in many human diseases including cancer, but also in ageing and chronic disease such as diabetes. Their intricate involvement in many cellular processes and pathways is being dynamically deciphered in many organs including the endometrium. This review summarizes our current knowledge on the topic of telomeres and telomerase and their potential role in providing plausible explanations for endometrial aberrations related to common gynaecological pathologies.

OBJECTIVE AND RATIONAL: This review outlines the recent major findings in telomere and telomerase functions in the context of endometrial biology. It highlights the contemporary discoveries in hormonal regulation, normal endometrial regeneration, stem cells and common gynaecological diseases such as endometriosis, infertility, recurrent reproductive failure and endometrial cancer (EC).

SEARCH METHODS: The authors carried out systematic PubMed (Medline) and Ovid searches using the key words: telomerase, telomeres, telomere length, human telomerase reverse transcriptase, telomeric RNA component, with endometrium, hormonal regulation,
endometrial stem/progenitor cells, endometrial regeneration, endometriosis, recurrent miscarriage, infertility, endometrial hyperplasia, EC and uterine cancer. Publications used in this review date from 1995 until 31st June 2016.

**OUTCOMES:** The human endometrium is a unique somatic organ, which displays dynamic telomerase activity (TA) related to the menstrual cycle. Telomerase is implicated in almost all endometrial pathologies and appears to be crucial to endometrial stem cells. In particular, it is vital for normal endometrial regeneration, providing a distinct route to formulate possible curative, non-hormonal therapies to treat chronic endometrial conditions. Furthermore, our current understanding of telomere maintenance in EC is incomplete. Data derived from other malignancies on the role of telomerase in carcinogenesis cannot be extrapolated to EC because unlike in other cancers, TA is already present in proliferating healthy endometrial cells.

**WIDER IMPLICATIONS:** Since telomerase is pivotal to endometrial regeneration, further studies elucidating the role of telomeres, telomerase, their associated proteins and their regulation in normal endometrial regeneration as well as their role in endometrial pathologies are essential. This approach may allow future development of novel treatment strategies that are not only non-hormonal but also potentially curative.

**Key words:** endometrium / telomerase / telomere / stem cells / endometriosis / endometrial cancer / infertility / recurrent miscarriage / progesterone / estrogen

---

### Introduction

All eukaryotic chromosomal ends consist of specialized heterochromatin nucleoprotein complexes, termed telomeres, containing repeated nucleotide sequences ((TTAGGG)_n) and associated specific proteins (Blackburn and Gall, 1978). The intact telomeres prevent the chromosomal ends from being recognized as DNA strand break and protects the loss of genomic DNA as well as end-to-end fusion and degradation of chromosomes. Telomeric DNA is lost with each round of DNA replication (Olovnikov, 1971; Watson, 1971; Lundblad, 2012) and shortening of telomeres beyond a critical length results in a permanent cell cycle arrest. This is due to initiation of sustained DNA damage signalling, resulting in activation of either senescence or apoptosis pathways (Blackburn and Gall, 1978; Blackburn et al., 2015). Telomere shortening and telomerase dysfunction are therefore implicated as universal features of cellular senescence and ageing as well as the age-related decrease in tissue regeneration and lifespan restriction in long lived mammals (Djojosubroto et al., 2003; Mikhelson and Gamaley, 2012).

The action of the reverse transcriptase enzyme telomerase is the main mechanism that counteracts telomere shortening in cells. Human cells such as embryonic stem cells, germ line cells and cancer cells with unlimited replicative capacity express high levels of telomerase activity (TA) which maintains and elongates telomeres, compensating for telomeric erosion (Counter et al., 1992; Yang and Huang, 2014; Meena et al., 2015). In contrast, adult stem/progenitor cells (SPCs) have the potential to up-regulate telomerase but these cells also undergo telomere shortening with age (Hiyama and Hiyama, 2007; Flores et al., 2008; Rane et al., 2016). Most human somatic cells do not express significant levels of TA (Opsresko and Shay, 2016) and age-related telomere shortening is commonly described in many human proliferative tissues (Djojosubroto et al., 2003) while telomere shortening in post-mitotic tissues is negligible (Benetos et al., 2011). Therefore, most work on the functional relevance of telomerase is confined to the aforementioned specialized cells that express telomerase, such as cancer and stem cells.

The human endometrium is a unique organ in terms of regeneration and ageing. It is a dynamic somatic tissue that undergoes repetitive monthly cycles of growth, differentiation, shedding and regeneration throughout a woman’s reproductive lifespan. These endometrial cycles of are regulated by ovarian steroid hormones (Hapangama et al., 2015).

Every month, the endometrium grows from 1 mm in thickness, at the end of the menstrual shedding, to 15 mm in thickness, measured in the mid-secretory phase of the cycle (Dallenbach-Hellweg and Dallenbach, 2010), thus the endometrial regeneration capacity is unparalleled amongst other adult tissues. At the menopause, with the cessation of ovarian steroid hormone synthesis, the endometrium becomes proliferatively quiescent. However, a fully functional endometrium can be regenerated from the remaining thin postmenopausal endometrium with the provision of exogenous ovarian steroid hormones (Paulson et al., 2002). Thus, it is the only female reproductive organ not showing irreversible age-related changes. Although it is a somatic organ, endometrium expresses dynamic TA associated with the menstrual cycle. Therefore, the apparent endometrial age-defiance might include a physiological regulation of telomeres and telomerase distinct from other human tissues.

This review focuses particularly on recent findings in endometrial telomere and telomerase biology in the context of the inexhaustible proliferative and regenerative capacity of the human endometrium. This may provide an explanation for the seemingly eternal ‘youthfulness’ retained by the endometrium throughout a woman’s life when compared with other female reproductive organs. Furthermore, there is mounting evidence that telomerase and telomere dysfunctions might play important roles in endometrial pathologies.

### Method

We performed systematic PubMed (Medline) and Ovid searches using key words: telomerase, telomeres, telomere length (TL), telomerase reverse transcriptase (TERT), telomeric RNA component (TERC), with endometrium, endometrial SPCs, endometrial regeneration, endometriosis, recurrent miscarriage, infertility, endometrial hyperplasia (EH), endometrial cancer (EC) and uterine cancer. All studies investigating telomerase or telomere biology in endometrium in women or animals or respective cell lines, either primary cells or tissue explants in culture, and published from 1995 until 30th July 2016, were considered. Further manuscripts published before 1995 were also reviewed for specific topic areas and are included as appropriate.
Telomeres

Structure

The mammalian telomere complex consists of a tandemly repeated telomeric DNA sequence 5′(TTAGGG)n and its complementary strand. This is, followed by a short (35–600 nucleotide) single-stranded 3′ guanosine-rich protruding overhang, known as the G-tail (Royale 2006; Blackburn et al., 2015). Telomeres are associated with a complex of six well-described shelterin proteins: Telomeric Repeat Factor 1 and 2 (TRF1, TRF2), Repressor/Activator Protein 1 (RAP1, encoded by TERF2IP gene), TRF1-Interacting Nuclear Protein 2 (TIN2), Tripeptidyl Peptidase I (TPP1) and Protection of Telomeres I (POT1). TRF1 and TRF2 bind directly to the double-stranded telomeric sequence, and POT1 binds the single-stranded overhang; these proteins are therefore telomere DNA binding proteins. They interact with and bind to the remaining three shelterin proteins: TIN2 to TRF1, RAP1 to TRF2 and TPP1 to POT1.

The single-stranded overhang forms a D-loop (displacement) (Griffith et al., 1999) that prevents the access of telomerase outside of late S-phase when the overhang becomes accessible (Fig. 1A) (de Lange 2005; Palm and de Lange 2008; Shay 2016). In addition, the whole telomere forms a large duplex structure (T-loop) via the strand invasion from the 3′ single-stranded overhang (Griffith et al., 1999; Blackburn et al., 2000) providing proper telomere capping. The T-loop size is believed to be proportional to the length of the respective telomere (Cimino-Reale et al., 2001). Thus, in addition to telomere shortening, the dysfunction of telomere capping can also initiate a DNA damage response (DDR) (Griffith et al., 1999; Yoo and Chung 2011; Bodvarsdottir et al., 2012).

The shelterin complex is ubiquitously expressed and remains associated with telomeres during the cell cycle (Royale 2006; Takai et al., 2011). In addition to the six shelterin proteins, there are various additional proteins (e.g. NBS1/MRE11/Rad50, tankyrase, PinX1, Ku) located at the telomere that are involved in DDR and repair processes but also have non-telomeric functions (Kuimov 2004; De Boeck et al., 2009).

Telomere shortening

Telomere shortening with age is a general observation in human proliferating tissues. In cell culture, with each cell division, about 20–50 base pairs (bp) of telomeric DNA is lost due to the end replication problem (Olovnikov, 1973; Watson, 1972). This term describes the fact that DNA polymerases can only synthesize in 5′–3′ direction and thus can only synthesize the leading DNA strand un-interrupted. The lagging strand is synthesized by a series of Okazaki fragments which requires the help of short RNA primers and that are finally ligated together by ligases. However, at the very end of the lagging strand, the terminal RNA primer is removed resulting in the 3′ overhang and loss of the DNA in the next round of replication (Watson, 1972; Olovnikov, 1973; Blackburn, 1984; Lundblad, 2012; Mikhelson and Gamaley, 2012; Blackburn et al., 2015). In addition to the end replication, environmental conditions such as oxidative stress are an additional mechanism of telomere shortening (von Zglinicki et al., 1995; von Zglinicki, 2000). The progressive loss of mean TL is a hallmark of replicative senescence of proliferating cells while the amount of telomere shortening can vary in different tissues and organs during aging and disease conditions depending on cell proliferation (Benetos et al., 2011; Benetos et al., 2011) and oxidative stress (von Zglinicki et al., 2000). Human mean TLs are 12–15 kb at birth and shorten down to a minimal TL of around 5 kb when a DDR and cell cycle arrest are signalled, which can lead to cellular senescence (Kipling and Cooke, 1990; Calado and Dimitriu, 2013). Shorter telomeres in lymphocytes have been associated with mortality, disease and poor-survival as well as reproductive ageing in humans (von Zglinicki et al., 2000; Cawthon et al., 2003; Shay, 2016). Thus, TL in human peripheral blood monocytes (PBMCs) has been proposed as a useful biomarker for human ageing and disease (von Zglinicki et al., 2000; von Zglinicki, 2002; Bekarta et al., 2005).

Although the mean TL of PBMCs had been employed in age determination in forensic medicine, the veracity of this approach is questionable due to the fact that TL is also inherited. In addition, more subtle methods for TL measurements considering initial TL as well as telomere shortening rates have been proposed (Benetos et al., 2011; Benetos et al., 2013). TL shortening starts during early gestation in many human tissue types such as heart, kidney and brain due to the down-regulation of TA (Ulmer and Giudice, 1997; Ulmer et al., 1998, 2001) and fast postnatal organ-specific growth accounts for most of the observed differential organ-specific telomere shortening rates (Carneiro et al., 2016).

Functions of telomeres

Prevention of recognition of linear chromosomal ends as double-stranded DNA breaks

The shelterin complex and the telomeric loop structure prevent telomeres from being identified as a DNA break that would signal a DDR. TRFs and POT1 prevent DDR activation due to the formation of the T-loop (Palm and de Lange 2008); TRF2 prevents end-to-end fusion (Cesare and Karlseder 2012) and POT1 helps to prevent the single-stranded telomeric 3′ end from being recognized by the DDR complex by forming a displacement (D-) loop with the remaining double strand (Jacob et al., 2007; Yang et al., 2008; Baumann and Price, 2010; Kibe et al., 2010).

Protection of chromosome ends from degradation and end-to-end fusion

Telomeres protect chromosomal ends from degradation by nucleases. Different DNA damage checkpoint proteins act together with EXO1 and MRE11 nucleases to inhibit proliferation of cells undergoing telomere attrition (Keijzers et al., 2016; Xue et al., 2016). Without the protective capping structure of telomeres, chromosomal ends would fuse together and form anaphase bridges during mitosis leading to a fusion-breakage-fusion cycle. This process would greatly increase the risk of genomic instability and may result in tumourigenesis (Djojosubroto et al., 2003; Meena et al., 2015; Shay, 2016; Terali and Yilmazer, 2016).

Sentinels for DNA damage

Telomeres are more susceptible to DNA damage than genomic DNA (Petersen et al., 1998) due to their high guanine content (Henle et al., 1999; Wang et al., 2010) and lack of DNA repair mechanisms (Petersen et al., 1998). Telomere-associated DNA damage in the form
**Figure 1** Schematic illustration of the main telomerase subunits and their interaction with the telomere complex. (A) Illustration of the human telomere complex and telomerase (only one half of the dimeric holoenzyme complex is shown for clarity). Out of the shelterin proteins, telomere repeat binding factors 1 (TRF1) and 2 (TRF2) bind directly to the double-stranded telomeric sequence, and protection of telomeres protein-1 (POT1) binds to the single-stranded overhang; hence these are named telomere binding proteins and interact with remaining shelterin proteins TIN2 (binds to TRF1), RAP1 (binds to TRF2) and TPP1 (binds to POT1). Telomerase associated proteins NOP10, NHP2 and GAR1 form the H/ACA motif-of the human telomerase reverse transcriptase (hTERT) associated tetramer with dyskerin. (B) The four functional domains of hTERT: the telomerase N-terminal (TEN) domain has roles in recruiting telomerase to telomeres as well as telomeric repeat synthesis; telomeric RNA component (TERC)-binding domain (TBD) interacts with hTERC; and both the reverse transcriptase (RT) domain and C-terminal extension (CTE) contribute to the reverse transcriptase enzyme activity (Nakamura et al., 1997; Blackburn and Collins, 2011). (C) Diagram of the core elements of hTERC: 5′ region containing (A) the pseudoknot domain and (B) stem terminus element-loop that contains the 11 nucleotide RNA template and...
of TIFs (telomere dysfunction-induced foci) or TAFs (telomere-associated foci) is hardly ever repaired (d’Adda di Fagagna et al., 2003; Takai et al., 2003; Takai et al., 2011; Fumagalli et al., 2012; Hewitt et al., 2012). This telomere-associated damage can have the same function as critically shortened telomeres in signalling cell cycle arrest. As the ‘first responders’ to hazards of genomic instability, the damaged telomeric DNA initiates a sustained DDR, resulting in a cell cycle arrest and inducing senescence or apoptosis, thereby protecting the organism from dangerous genetic aberrations and mutations (Blackburn et al., 2015; Shay 2016). Telomeres have thus been proposed to be sentinels for DNA damage (von Zglinicki 2002) and epigenetic sensors of general stress in DNA metabolism (Cesare and Karlseder 2012).

Recruitment of telomerase and regulation of its access to telomeres
Shelterin (Fig. 1) has a dual role in recruitment of telomerase and blocking its access to telomeres (Smogorzewska and de Lange 2004; Palm and de Lange 2008; Nandakumar and Cech 2013; Zhang et al., 2013; Schmidt and Cech 2015). POT1 prevents telomerase accessing an intact telomere complex but after hetero-dimerization with TPP1, it allows telomerase to become active at telomeres and to extend the 3’ overhang in late S-phase (Wang et al., 2007; Zhang et al., 2013; Chu et al., 2016).

Regulation of gene transcription/telomere position effect
Telomeres may also regulate gene transcription via a telomere position effect (TPE) (Robin et al., 2014), whereby genes located close to the telomeres are transcribed at a reduced rate. This allows changeable epigenetic transcriptional repression permitting genes the ability to switch their transcription rate. TPE has been reported to affect the expression of genes involved in stress, growth and recognition by the immune system in various invertebrate organisms and in cultured human cells (Robin et al., 2014). Recently it has also been connected to human diseases (Stadler et al., 2013). Further work examining the role of TPE in gene regulation in human tissues and during telomere shortening is needed to unravel its involvement in endometrial diseases.

Non-telomeric functions of telomere-associated proteins
Some shelterin proteins also have non-telomeric, genomic binding sites that allow extra-telomeric functions, such as regulating transcription of various genes. For example, RAP1 has been shown to regulate female obesity, a function unrelated to telomeres (Martinez et al., 2013). Cell type, subcellular localization and development stage specific pathways may regulate the shelterin complex. TIN2 has been found in mitochondria (Chen et al., 2012) and a reduction in TIN2 expression inhibits glycolysis and reactive oxygen species production and enhances ATP levels and oxygen consumption in cancer cells. This suggests a link between some shelterin proteins and metabolic control, providing an additional mechanism by which telomeric proteins might regulate the cellular processes beyond their function at telomeres. Additional non-telomeric functions in embryonic development have also been described for TIN2 (Chiang et al., 2004).

Telomerase maintenance
Cells can maintain their telomeres via a telomerase dependent pathway or a telomerase independent alternative lengthening of telomeres (ALT) pathway (Brien et al., 1997; Bryan et al., 1997). Although activation of the latter pathway has been limited to particular types of cancers (sarcomas) and immortalized cell lines, there are suggestions that the ALT process may occur under physiological conditions in undifferentiated cells such as stem cells or even normal somatic cells (Neumann et al., 2013; Bojovic et al., 2015). There is a general consensus that in telomerase competent cells and in most normal cells, the ALT process is redundant and hence repressed (Henson et al., 2002). Therefore, in the context of the endometrium, ALT is less likely to be relevant and this review focuses mainly on telomerase dependent telomere maintenance.

Telomerase
Telomerase is a reverse transcriptase (RNA dependent DNA polymerase) that employs an integral RNA subunit harbouring a template sequence to add G-rich telomeric repeats to the 3’ single-stranded overhang of telomeres (Lingner et al., 1997). The telomerase holoenzyme has a dimeric structural configuration, where each half contains a human TERT (hTERT) and human TERC (hTERC) connected by a hinge region in the middle (Blackburn, 2011; Blackburn and Collins, 2011) (Figs 1B and C). The main components of the telomerase complex are hTERT, hTERC and dyskerin (DKC1) (Venteicher et al., 2009) (Fig. 1C). In addition, there are various telomerase associated proteins that interact with these core components (Fig. 1A). There are 184 telomeres and approximately 250 molecules of telomerase and in a cancer cell in late S-phase, when telomerase is actively recruited to telomere ends (Moody and Cech, 2006; Xi and Cech, 2014; Schmidt and Cech, 2015). When not active at telomeres, telomerase is localized to Cajal bodies in the nucleus for most of the cell cycle. After the telomerase/telomere interaction, every single telomerase activation event is thought to add 50–60 nucleotides to most telomeres in cancer cells with short TLs in vitro (Zhao et al., 2009; Schmidt and Cech 2015). Since the telomere lengthening action is limited to the nucleus, shuttling of the telomerase protein hTERT out of the nucleus prevents any telomeric extension. This shuttling is regulated by different domains on the hTERT protein; for example, a nuclear localization signal at amino acid residues 222–240 of hTERT (Chung et al., 2012) and a nuclear export signal (Seimiya et al., 2000) as well as a mitochondrial localization signal (Santos et al., 2004) have been described. Furthermore, recent data also suggest that the ability of telomerase in extending telomeres may be dependent on pH levels.

(C) the template boundary element (Theimer and Feigon, 2006). Both A and B domains are important for in vivo stability of hTERC and they interact with hTERT. The RNA stabilizing 3’ region contains (D) an H/ACA motif, which interacts with dyskerin or any of the other three H/ACA RNP components (NOP10, NHP2 and GARI), and (E) trans-activating domain containing CR4/5 C that also binds hTERT (Webb and Zakian, 2016). The template boundary element together with the 3’ end prevents DNA synthesis beyond the template (Feng et al., 1995; Fu and Collins, 2003; Kiss et al., 2010).
(Ge et al., 2016). Acidic pH (6.8) encourages preferential lengthening of short telomeres yet telomerase lengthens telomeres independent of their lengths at higher pH levels (7.2, 7.4).

Telomerase components
Telomerase reverse transcription activity has been demonstrated in an in vitro cell free system with just hTERT and hTERC (Weinrich et al., 1997). However, some compounds such as dyskerin actively associate with the telomerase complex in a cellular environment and are important for stability, maturation and function of the enzyme (Cohen et al., 2007).

Human telomerase reverse transcriptase
hTERT is the catalytic subunit of telomerase and is often the main rate-limiting factor for telomerase enzyme activity (Zhou et al., 2006; Zhang et al., 2013). The hTERT gene is located on chromosome 5p15.33 (Shay 2010) and consists of 16 exons and 15 introns spanning ∼35 kb (Cong et al., 1999). There are over 20 spliced variants of hTERT but only the wild type (full length protein, Fig. 1B) exhibits reverse transcriptase activity (Hrdlickova et al., 2012). The balance between the full lengths hTERT and its different splice variants has been shown to affect its function (Listerman et al., 2014; Radan et al., 2014). In addition to telomere maintenance, hTERT is implicated in increasing the anti-apoptotic capacity of cells, maintaining pluripotency of stem cells and regulating gene expression (for review see Saretzki, 2014).

Human telomeric RNA component
TERCs are species specific in size and sequence, but highly conserved in their structure and all contain a short sequence complementary to the telomeric TTAGGG hexanucleotide repeat sequence. Human TERC is relatively short (451 nucleotides (nt) compared with >1000 nt in yeast) (Theimer and Feigon 2006). The 3′ stabilizing element shares an H/ACA motif with small nucleolar and small Cajal body RNAs (snoRNA, scaRNA), and in turn associates with all four H/ACA RNP components, dyskerin, NOP10, NHP2 and GAR1 (scaRNA), and in turn associates with all four H/ACA RNP components, dyskerin, NOP10, NHP2 and GAR1 (Fig. 1A). hTERC is a non-coding RNA transcribed by RNA polymerase II (Gallardo and Chartrand 2008; Smekalova et al., 2012). It undergoes subsequent exonucleolytic cleavage up to the boundary formed by the H/ACA domain, meaning its co-transcriptional association with dyskerin is essential for stabilization, preventing further cleavage and nuclear retention (Feng et al., 1995; Fu and Collins 2003; Kiss, Fayet-Lebaron and Jady 2010). This H/ACA domain is mutated in dyskeratosis congenita, where the disease-associated hTERC variants impair hTERC accumulation. Disease-associated hTERC variants with sequence changes outside the H/ACA domain do not affect hTERC RNA processing or stability; they instead impose a catalytic defect (Fu and Collins 2003). The tetrameric complex of the accessory proteins dyskerin, NOP10, NHP2 and chaperone NAF1 (which later is replaced by GAR1) bind to hTERC and this association is crucial for normal TA.

Dyskerin
Dyskerin is an evolutionarily conserved 58 kDa, 514-amino-acid large protein (Knight et al., 1999). In humans, it is encoded by the DKC1 gene located on chromosome Xq28 (Cerrudo et al., 2015) and it is generally located in the nucleus. Dyskerin is an essential protein for cellular survival; thus DKC1 deletion lethality (Rocchi et al., 2013; Angrisani et al., 2014). In the context of telomerase, dyskerin plays an established role in the maintenance of telomere integrity by stabilizing hTERC in the telomerase holoenzyme that is assembled in Cajal bodies (Cohen et al., 2007; Gallardo and Chartrand 2008; Gardano et al., 2012). Dyskerin is the only component to co-purify with active, endogenous human telomerase (Gardano et al., 2012). Loss of dyskerin binding leads to hTERC degradation and reduction in TA in vivo (Shukla et al., 2016). Furthermore, dyskerin has other non-telomerase associated functions essential to elementary cellular events such as mRNA translation, growth and proliferation. Dyskerin may regulate these functions via directing the isomerization of specific uridines to pseudouridines by acting as a catalytic pseudouridine synthase and by acting through the snRNA-derived mRNA regulatory pathway, thus affecting different biological processes (reviewed in Angrisani et al., 2014).

Other accessory proteins of telomerase
Apart from NOP10, NHP2 and GAR1 which form the H/ACA motif-associated tetramer with dyskerin (Fig. 1), there are a plethora of other proteins (some listed in Table 1) associated with telomerase with roles including: assembly, processing of telomerase, localization and accessibility to telomeres. In addition to these proteins, telomerase interacts with many others which are required for the formation of the appropriate structure and its stabilization, however their importance in TA is unknown (Smekalova et al., 2012). It is important also to appreciate the close relationship of telomerase with many cell cycle regulating, tumour suppressor, pluripotency and epithelial–mesenchymal transition (EMT) related proteins and pathways, such as Wnt/β-catenin, Cyclin D1, BCL-2, OCT-4, p53, EGFR, etc. (Ding et al., 2011; Listerman et al., 2014; Tang et al., 2016; Xue et al., 2016). Interestingly, recent data has suggested that the DDR kinases Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangectasia and Rad3-Related Protein (ATR) are required to recruit telomerase to telomeres via a TRF1 regulated pathway (Tong et al., 2015), while a central role of the ATM pathway in regulating telomere addition has been further highlighted (Lee, Bohrson, et al., 2015). In yeast, there exists a counting mechanism involving the shelterin RAP1, which prevents ATM activating/activating telomerase on long telomeres thereby regulating TL (Yuan et al., 2013). However, whether a similar feedback mechanism exists in human cells is not yet known (Runge and Lustig, 2016). These findings are only beginning to unravel the intricate cellular pathways that are converging to regulate telomerase and telomere biology.

Functions of telomerase
Telomere maintenance
In eukaryotic cells, TA counteracts the end-replication problem and elongates the 3′ single strand in the absence of a DNA template. The subsequent replication of the complementary C-rich strand then will be possible by the conventional DNA replication in the next S-phase. The human telomerase complex consisting of hTERT and hTERT is targeted to telomeres specifically in the late S-phase of the cell cycle (Hug and Lingner 2006). Recent work has suggested that hTERT remains bound to hTERC for most of the cell cycle (Vogang and Collins 2015). The telomerase holoenzyme Cajal body-associated protein, TCAB1, is released from hTERT during cell cycle progression in mitotic cells coincident with TCAB1 de-localization from Cajal bodies (Vogang and Collins 2015). This observation proposes that
TCAB1 and hTERC association may license the catalytically active hTERT–hTERC holoenzyme for recruitment to telomeres in the G1 phase of the cell cycle. TRF1 is the shelterin protein that is primarily responsible for regulation of an efficient replication of telomeric DNA (Seif et al., 2009). Apparently not all telomeres are required to be elongated by telomerase in each DNA replication round but there might be preferential lengthening of the shortest telomeres in telomere active cells to ensure all the TLs remain above a critical length that would otherwise initiate activation of apoptotic and cell cycle arresting pathways (Fakhoury et al., 2010; D’Souza et al., 2013). The exact mechanism by which telomerase differentially extends telomeres with various lengths is still not well understood.

### Table 1 Telomerase associated proteins.

| hTERT associated proteins | Function |
|---------------------------|----------|
| P23, hsp90                | Assist in ribonucleoprotein (RNP) assembly (Elmore et al., 2008). |
| Protein 14-3-3            | • Regulates signal transduction and apoptosis  
                          | • Prevents nuclear export of hTERT  
                          | • Regulates localization of the hTERT binding partners (Xi and Cech, 2014) |
| DHX36 (DEAH-Box Helicase 36) Protein | • Stabilizes hTERT  
                          | • Corrects positioning of the template domain of hTERT (Sexton and Collins, 2011) |
| Nuclear VCP-Like protein 2 (NVL2) | • Knockdown reduces telomerase activity (Her and Chung, 2012) |
| Pontin and reptin          | • Facilitate assembly of the minimally active enzyme consisting of TERT, TERC and dyskerin (Huber et al., 2008) |

| hTERC associated proteins | Function |
|---------------------------|----------|
| GAR1, NAP57, NOP10, NHP2 (members of H/ACA ribonucleoproteins complex) | • Associated with stability, accumulation, maturation and localization of hTERC  
                          | • Regulate cell proliferation (Wang and Meier, 2004) |
| Dyskerin (DKC1, a member of H/ACA ribonucleoproteins complex) | • Stabilizes TERC  
                          | • Reduces genetic instability  
                          | • Effective RNA pseudouridylation loss of dyskerin arrests the cell cycle (Ashbridge et al., 2009; Egan and Collins, 2010) |
| A1, UP1                   | Help in accessibility of telomerase to telomeres (Fiset and Chabot, 2001; Nagata et al., 2008) |
| TEP1 (telomerase protein component 1) | Not essential for telomerase activity or telomere length maintenance in vivo, essential for telomere replication (Harrington et al., 1997) |
| La antigen                | Direct and specific interaction between La and TERC influences telomere homeostasis (Aigner et al., 2003) |
| STAU (human homologue of staufen), RPL22 (Ribosomal Protein L22) | Assist in telomerase processing, localization and telomerase assembly (Le, Stemmglanz and Greider, 2000) |
| TCAB1 (telomerase and Cajal body protein 1, encoded by WRAP53) | May license the catalytically active hTERT–hTERC holoenzyme for recruitment to telomeres (Venteicher et al., 2009) |

TCAB1 and hTERC association may license the catalytically active hTERT–hTERC holoenzyme for recruitment to telomeres in the G1 phase of the cell cycle. TRF1 is the shelterin protein that is primarily responsible for regulation of an efficient replication of telomeric DNA (Seif et al., 2009). Apparently not all telomeres are required to be elongated by telomerase in each DNA replication round but there might be preferential lengthening of the shortest telomeres in telomerase active cells to ensure all the TLs remain above a critical length that would otherwise initiate activation of apoptotic and cell cycle arresting pathways (Fakhoury et al., 2010; D’Souza et al., 2013). The exact mechanism by which telomerase differentially extends telomeres with various lengths is still not well understood.

**Non-telomeric functions of the telomerase component hTERT**

Although telomere protection/lengthening is the most widely studied function of telomerase, there has been a considerable amount of evidence on non-telomeric functions of the protein subunit hTERT, such as promoting cellular proliferation/growth, survival, retaining an undifferentiated status, as well as increasing motility and metabolism (reviewed in Saretzki, 2014; Terali and Yilmazer, 2016). Recent evidence also suggests that hTERT binds to and stimulates ribosomal DNA transcription, particularly under hyper-proliferative conditions (Gonzalez et al., 2014). Protection of mitochondrial function under oxidative stress has been proposed as an important role of hTERT in various cell types, it was associated with reduction in oxidative stress and sensitivity to apoptosis as well as with a reduction in DNA damage (Ahmed et al., 2008; Singapoli et al., 2013). While initial studies on the beneficial role of mitochondrial hTERT were conducted mainly in vitro in cell culture, recent data describe beneficial effects also in vivo, for example, in vascular function (Beyer et al., 2016). Telomerase may interact with various well-established proliferative pathways including EGFR signalling (Smith et al., 2003), MYC and Wnt/β-catenin pathways (Park et al., 2009; Hoffmeyer et al., 2012). Telomerase has been shown to promote cell survival by blocking the death receptor (Dudognon et al., 2004) as well as by down regulating pro-apoptotic genes such as BAX and BCL-2 (Del Bufalo et al., 2005; Massard et al., 2006) in addition to suppressing mitochondrial and endoplasmic reticulum stress induced cell death (Zhou et al., 2006). Telomerase inhibition in stem cells has induced differentiation and loss of pluripotency genes, suggesting a role as a pluripotency gene in embryonic stem cells maintaining an undifferentiated status (Saretzki et al., 2008; Yang et al., 2008; Liu et al., 2013).

**The endometrium**

Human endometrium lines the uterine cavity and is organized into two functionally distinct layers: the superficial functionalis and deeper basalis (Valentijn et al., 2013); (Hapangama, Kamal and Bulmer 2015). The transient, exquisitely hormone responsive, functionalis exists only during the reproductive life of a woman, whereas the permanent, relatively hormonally unresponsive basalis layer persists.
throughout her whole life. The endometrial menstrual cycle is an exclusive phenomenon to upper order primates and is regulated by ovarian steroid hormonal signals (Hapangama et al., 2015; Kamal et al., 2016; Kamal et al., 2016) (Fig. 2A).

Endometrium is the primary target organ for ovarian steroid hormones and the endometrial cell cycle is intricately regulated by them. The reproductive lifespan of a woman is dictated by ovarian function. It commences with menarche and finishes with menopause. During that period, an average woman endures about 400 menstrual cycles in which the functionalis layer of the endometrium undergoes a well-defined cycle of proliferation, differentiation and menstrual shedding followed by regeneration. Ovarian steroid hormones regulate this endometrial cycle via their cognate receptors (reviewed in Hapangama et al., 2015; Kamal et al., 2016). It is generally accepted that estrogen is the trophic hormone of the endometrium, where it induces cellular growth and proliferation; while progesterone influences cellular differentiation and counteracts proliferation and other estrogenic effects (Hapangama, 2003). The third ovarian steroid hormone, androgen, is also postulated to impact the endometrial cycle, yet unlike the aforementioned hormones, the exact details of androgenic regulation of the endometrium are yet to be fully elucidated. The huge regenerative potential seen with the monthly endometrial cycle is unparalleled by other tissues, and the exact reason for this menstrual shedding, which is biologically a very expensive process, is yet unknown.

**Telomerase and telomeres in endometrial tissue: functional relevance**

TA is high in the premenopausal endometrial functionalis (Kyo et al., 1997) (Fig. 2C and D). Its dynamic changes regulated by the ovarian cycle are well established and correlate with glandular proliferation (Williams et al., 2001; Hapangama et al., 2008; Hapangama et al., 2009). Our recent work further demonstrates similar dynamic changes in the mean endometrial TLs across the menstrual cycle (Valentijn et al., 2015) (Fig. 2B). Once the ovarian hormone production has ceased, the relatively quiescent postmenopausal endometrium expresses low levels of TA (Brien et al., 1997; Tanaka et al., 1998).

When examining distinct cellular compartments within the endometrium, stromal cells, regardless of the cycle phase, maintain longer TLs compared with the epithelial cells. However, they demonstrate absent or significantly lower TA (Fig. 3A–C) and hTERC expression compared with the epithelial cells (Tanaka et al., 1998; Yokoyama et al., 1998; Valentijn et al., 2015). (Vidal et al., 2002). Data from a previous study which employed in situ assessment of endometrial TLs also demonstrated that glandular epithelium of the endometrial functionalis possesses the shortest TL (Cervello et al., 2012) (Fig. 3D). Furthermore, the proliferating endometrial epithelial cells have the highest TA that correlates negatively with TL (Valentijn et al., 2015) (Fig. 3B). This suggests that in the epithelial cells, the high TA preferentially preserves the

![Figure 2](https://academic.oup.com/humupd/article-abstract/23/2/166/2691292/figure2)

**Figure 2** Correlation of typical ovarian hormonal changes with the observed changes in endometrial telomerase activity (TA), mean telomere length (TL) and endometrial hTERT protein expression. (A) Estrogen (green line) and progesterone (orange line) (ovarian hormones) show typical cyclical variations during the menstrual cycle in premenopausal women. (B) Endometrial TA increases steadily under the influence of estrogen in the proliferative phase, whereas the levels plummet in the progesterone-dominant secretory phase of the cycle (Kyo et al., 1997; Saito et al., 1997; Williams et al., 2001; Hapangama, Turner, Druy, Quenby, et al., 2008; Hapangama et al., 2009). (C) Our recent work further demonstrates similar dynamic changes in the mean endometrial TL across the menstrual cycle (Valentijn et al., 2015). (D) In full thickness endometrial tissue sections, hTERT protein expression studied with immunohistochemistry employing a monoclonal mouse anti-human telomerase antibody (ab27573, Abcam, Cambridge UK), detection with ImmPRESS anti-mouse/rabbit polymer and visualization with ImmPACT DAB (Vector Laboratories, Peterborough, UK). Positive hTERT staining was observed in functionalis and basalis epithelial cells in the proliferative phase but the brown positive staining is limited to the basalis epithelium in the secretory phase. Magnification x200, Scale bar 10 μm.
short telomeres in order to avoid a critically short length. In rodents, estrogen increases the pH in the uterine fluid, while progesterone has the opposite effect (Chinigarzadeh et al., 2016). It can therefore be speculated that low pH in the proliferative phase may preferentially direct telomerase function to the short telomeres in endometrial epithelial cells. This is in accordance with the recent evidence regarding pH dependent telomerase function (Ge et al., 2016). Further studies would be required to fully investigate and confirm this possibility.

Moreover, endometrial hTERT may have extra-telomeric functions. Direct in vitro inhibition of TA with the TERC inhibitor ‘imetelstat’ inhibited endometrial cell proliferation and disrupted gland formation by healthy epithelial cells (Valentijn et al., 2015). In contrast, overexpressing hTERT in endometrial stromal cells did not increase cell proliferation rate or hormone responsiveness (Barbier et al., 2005) similar to the other non-endometrial fibroblasts (Ahmed et al., 2008). Thus, there might be a specific co-regulation of TA and proliferative capacity limited to the endometrial epithelial cells or to epithelial cells in general. Other groups have found a correlation between TA in ovarian granulosa cells and their proliferation and differentiation status which is also under the control of growth factors and steroid hormones, similar to endometrial epithelium (Chronowska 2012). Importantly, although it has been possible to immortalize benign endometrial stromal cells by overexpressing hTERT (Krikun et al., 2006), immortalization of endometrial epithelial cells using a similar process has not been equally successful. This might be due to the fact that epithelial cells are likely to require an additional inhibition of the p16INK4a tumour suppressor in order to be immortalized by hTERT overexpression (Kiyono et al., 1998; Farwell et al., 2000; Shao et al., 2008; Novak et al., 2009).

The previously reported immortalization of benign human endometrial epithelial cells with hTERT overexpression (Kyo et al., 2003) has not been successfully replicated. This is an important fact, as the only other supposedly immortalized benign endometrial epithelial cell line that was generated by telomerase overexpression (Hombach-Klonisch et al., 2005) was later confirmed to be the misidentified breast cell line MCF-7 (Korch et al., 2012). This particular cell line was widely available to several groups leading to many publications.
transcriptional activation by ligand-activated ER (King et al., 2010). Unfortunately, the reportedly immortalized epithelial cell line generated by Kyo and colleagues (Kyo et al., 2003) has not undergone similar scrutiny and has not been available to other groups for further confirmatory studies for authenticity. In summary, we conclude that there are fundamental differences in telomerase function between endometrial epithelial and stromal cells. The mere presence of telomerase may result in a survival advantage for stroma, while epithelial cell proliferation may be regulated by telomerase. Additional factors than TA seem to be necessary for the long term survival and immortalization of epithelial cells in culture.

We are just beginning to understand the importance of extra-telomeric functions of telomere and telomerase components; for example, RAPI has an extra-telomeric function on strand cell decidualization in the rat endometrium (Kusama et al., 2014). Further examination of extra-telomeric functions of telomerase/telomeric proteins in the endometrium is warranted to further reveal their interplay with other cell cycle regulators specific to the endometrium.

Hormonal regulation of telomerase in epithelial cells

TA can be regulated at multiple levels, e.g. transcription, splicing, epigenetic and post-translational modification (reviewed in Fojtova and Fajkus, 2014; Lewis and Tollefsbol, 2016; Akinlar et al., 2016). Most human somatic tissue has absent or low levels of TA which is tightly regulated compared with the high and easily detectable levels seen in cancer cells and in germ line/stem cells. Thus, the initial studies on telomerase regulation were conducted in the context of developmental or stem cell biology or in cancer cells (reviewed in Batista, 2014; Huang et al., 2014; Gladych et al., 2011). However, in this review, we focus primarily on the hormonal regulation of telomerase at a normal, physiological level in the endometrium which is pivotal to its function.

Estrogen
Early work on the hormone responsive breast cancer cell line MCF-7 showed that estrogen up-regulates TA and hTERT gene expression via direct and indirect effects on the hTERT promoter (Kyo et al., 1999). Gel shift assays on MCF-7 cells further revealed that there is an imperfect palindromic estrogen-responsive element in the hTERT promoter that specifically binds to estrogen receptor (ER) and is responsible for transcriptional activation by ligand-activated ER (Kyo et al., 1999). Further confirmation of 17β-estradiol (E2) induced transcription of hTERT vio ERα was also reported in various other cell types, including ovarian epithelial cells (Kimura et al., 2004; Gladych et al., 2011), ovarian stromal cells (Misti et al., 2000), mesenchymal stem cells (MSCs) (Cha et al., 2008) and human umbilical vein endothelial cells (Hiyama and Hiyama 2007). Although ChIP assays in prostate cells suggested a recruitment of both ER subtypes to the hTERT promoter, its induction by ERβ in other cells remains controversial (Nanni et al., 2002).

There is some evidence that longer exposure to endogenous estrogen (length of reproductive years of life) might correlate with greater TLs and TA in PBMCS (Pines 2013). In other words, longer TLs seem to be present in different tissues and may be associated with longer reproductive life. E2 increased TA and TERT mRNA in heart, liver and brain tissue in an ovarioctomized rat model (Cen et al., 2015). However, mature peripheral T cells do not respond to E2 with changes in expression or function of telomerase (Benko et al., 2012) suggesting that the effect of estrogen on telomerase is tissue/cell specific. Finally, the relative longevity of women compared with men has been speculated to be related to the effects of estrogen induced telomerase on telomere protection (Leri et al., 2000; Calado et al., 2009; Barrett and Richardson 2011; Gopalkrishnan et al., 2013; Cen et al., 2015). More active telomerase was found in cardiac myocytes from female rats which seems to correspond to higher myocyte numbers in older women compared to myocyte loss in older men (Leri et al., 2000). Others have suggested that reduction of oxidative stress by estrogens may result in longer telomeres in tissues such as brain and liver (Vina et al., 2005). Greater female longevity is suggested to possibly be connected to the female exposure to estrogens (Muezzinler et al., 2013). However, a recent longitudinal study reports a higher rate of PBMC TL attrition in the premenopausal period than in the postmenopausal period (Dalgaard et al., 2015) with the authors proposing the opposite effect of estrogen on leucocyte turnover and menstrual bleeding. Thus, the influence of estrogen on TL and female longevity is still controversial. There is in vivo and in vitro evidence suggesting that estrogens are able to induce TA and hTERT expression in the endometrium (Tanaka et al., 1998; Kyo et al., 1999; Vidal et al., 2002). In contrast, postmenopausal endometrium and endometrium treated with anti-estrogen drugs exhibited decreased TA (Tanaka et al., 1998). Furthermore, long term treatment with clinically relevant doses of conjugated E2 increased TERC expression preferentially in endometrial glands of ovarioctomized female cynomolgus macaques (Macaca fascicularis) (Vidal et al., 2002). Increased TERC levels also correlate with higher proliferation and progesterone receptor expression in the endometrium of treated animals (Vidal et al., 2002). Both of these parameters are known to be regulated by estrogen in the endometrium (Hapangama et al., 2015). In the ER positive, hormone responsive endometrial epithelial adenocarcinoma cell line (Ishikawa cells), E2 induced TA and hTERT mRNA levels via a MAPK dependent pathway in an ERα dependent fashion (Zhou et al., 2006). In contrast, isolated primary epithelial cells (Tanaka et al., 1998) or intact endometrium in short-term explant culture did not show a significant response to E2 on TA (Valentijn et al., 2015). Conversely, co-cultured primary endometrial epithelial and stromal cells responded to E2 or to a mitogenic fibroblast growth factor stimulus, suggesting that the E2 effect on telomerase induction may be enhanced or mediated by stroma and/or the duration of E2 treatment (Otsita et al., 2004).

Progesterone
Although progesterone has diverse effects on hTERT mRNA expression in progesterone receptor (PR) expressing breast and EC cell lines, the mechanisms by which hTERT expression is regulated by progesterone appear to be complex. The hTERT promoter lacks a canonical progesterone-responsive element (Wang et al., 2000), therefore classical PR mediated direct effects are less likely. The role of recently described progesterone receptor membrane components 1 and 2 on telomerase regulation has not yet been demonstrated (Bunch et al., 2014). In a breast cancer cell line, synthetic progesterogen, medroxyprogesterone acetate inhibited hTERT mRNA transcription even in the presence of estrogen (Wang et al., 2000; Lebeau et al., 2002) and arrested cells in the late G1 phase (Lebeau et al., 2002) with the induction of p21 (Lange et al., 1999; Wang et al., 2000). There is also evidence for cell cycle-dependent regulation of
telomere synthesis and telomerase gene expression in healthy hormone responsive human cells by progesterone (Wang et al., 2000; Tomlinson et al., 2006).

Since endometrial TA, hTERT mRNA/protein and hTERC levels reach their nadir during the progesterone-dominant mid-secretory phase, progesterone is thought to negatively regulate endometrial telomerase (Williams et al., 2001; Hapangama et al., 2008; Hapangama et al., 2009; Valentinj et al., 2015). The shortest endometrial TLs were also measured in the mid-secretory phase, indicating a telomere lengthening/maintenance function for endometrial TA (Valentinj et al., 2015). Exogenous progesterone administration is known to inhibit endometrial epithelial proliferation (Kurita et al., 1998; Shimizu et al., 2010) and we have recently shown this progesterone-induced decreased endometrial cell proliferation to be associated with a significant decrease in TA (Valentinj et al., 2015). Interestingly, the inhibition of TA in the progesterone-dominant secretory phase is associated with an induction of endometrial p21 and corresponds to a non-DNA damage induced cell cycle arrest function of p21 (Toki et al., 1998; Yoshimura et al., 2007). We can speculate that progesterone induced telomerase suppression might result in short endometrial epithelial telomeres (Hapangama et al., 2009; Valentinj et al., 2015) and perhaps influence changes in the endometrial epithelial cell cycle via p21 induction (Aix et al., 2016). Taken together, the hTERT gene may be a target of progesterone and the well-established progesterone induced down-regulation of the endometrial cell cycle may involve telomerase.

Androgens

Androgens such as dihydrotestosterone (DHT) induced TA at the G1 phase of the cell cycle in the androgen sensitive prostate cancer cell line LnCAP (Thelen et al., 2004). However, there was no modulation of TA by androgens in either androgen insensitive prostate cancer cell lines (TSU-Pr1, DU145) or in normal human prostate cells (Soda et al., 2000). In a recent study in men, serum DHT and E2 levels were shown to correlate with TL in PMBC, suggesting that both hormones may have a synergistic influence on TA (Yeap et al., 2016). However, caution should be taken when interpreting this observation as the authors have not demonstrated a direct regulatory effect. Oral treatment with Danazol (a synthetic steroid with weak androgenic properties) for 2 years resulted in universal leucocyte telomere elongation in both male and female patients with diseases such as bone marrow failure, liver cirrhosis and pulmonary fibrosis known to involve telomeres (Townesley et al., 2016). The intra-cellular metabolism of testosterone to estrogens is well described (Sasano et al., 2008). Androgens appear to regulate telomerase expression and activity mainly by aromatization of testosterone to estrogens through ERα in normal peripheral blood lymphocytes and human bone marrow-derived CD34(+) cells in vitro (Calado et al., 2009). Therefore, it is difficult to clearly ascertain if the observed effects of androgenic compounds were related to the direct effects on the androgens receptor (AR) or indirectly mediated via ER.

Andrometrium expresses AR yet the direct specific effects of androgens in normal endometrium are only beginning to be understood.

There is no published work yet examining the effects of androgens in endometrial telomerase regulation.

Other hormones relevant for the endometrium

Melatonin appears to regulate hTERT and hTERC expression in MCF-7 cells (Leon-Blanco et al., 2004) while dexamethasone reduced TA through the inhibition of TERT expression before induction of apoptosis (Akiyama et al., 2002). In contrast, hydrocortisone did not affect TA in human leucocytes (Calado et al., 2009). Therefore, the evidence for other non-ovarian steroidal hormones having a potential regulatory function of endometrial telomerase is limited and they will not be further discussed in this review.

Endometrial stem cells and telomerase

The involvement of SPCs in the endometrial regenerative process has been suggested for a long time (Priianishnikov 1978). After menstrual shedding, a new functionalis layer is thought to regenerate from the remaining SPC rich basalis (Valentinj et al., 2013; Hapangama et al., 2015) and SPCs in many other tissues have the potential to activate telomerase (Hiyama and Hiyama 2007). Interestingly, the available evidence for the differences in TA between the endometrial basalis and the functionalis is controversial. A study in which different endometrial layers were crudely isolated by scraping (using a curette or a scalpel) suggested that TA is lower in the basalis (Bonatz et al., 1998); whereas isolated basalis epithelial cells, identified by expression of the surface marker SSEA1 from primary endometrial epithelial cells in short-term culture, showed higher TA than functionalis epithelial cells (Valentinj et al., 2013). Our study examined only sorted endometrial epithelial cells and telomerase expression and TA were limited mainly to the epithelial cells (Tanaka et al., 1998; Yokoyama et al., 1998; Valentinj et al., 2015). Therefore both studies should have demonstrated similar results. The reasons for this contradictory observation could be due to the fact that epithelial SPC cells are likely to be activated during isolation and cultivation in the latter study, which removed the epithelial SPC cells from its niche, a process known to induce telomerase (Engelhardt et al., 1997). Furthermore, the presumed basalis tissue obtained by scraping the myometrium in the former study might have contained a higher proportion of myometrial tissue with low TA levels.

The available evidence suggests that the endometrium contains multiple progenitor cell populations. Cells with some stem cell properties have been isolated from the human endometrium expressing phenotypical markers of epithelial, stromal, leucocyte and vascular origin (Chan et al., 2004; Masuda et al., 2010; Cervello et al., 2011). Freshly isolated undifferentiated side population cells containing all these primitive cell types from human endometrium also expressed TA (Cervello et al., 2011). The presence of TA in the most widely characterized and studied endometrial SPC cell subtype, the endometrial stromal (mesenchymal) SPCs (Gargett et al., 2016), is yet to be fully described. Human MSCs (hMSC) from other locations are known to have negative or very low TA (Zimmermann et al., 2003; Tichon et al., 2009; Ogura et al., 2014). However, there is a report suggesting that early passages of endometrial stromal SPCs isolated...
on the basis of their expression of the putative MSC marker CD146 express hTERT protein and mRNA but the authors did not measure TA (Yang et al., 2011). Furthermore, isolated primary endometrial stromal cells had low but measurable TA in our recent study (Valentijn et al., 2015). Our unpublished data also suggest that TA in isolated primary stromal cells positively correlated with mean TLs, suggesting that telomerase expression may have a telomere lengthening function in these cells (Fig. 3). It will be interesting in the future to confirm these preliminary findings and examine the functional relevance of TA to TL in the different endometrial SPC subtypes. However, the low amount of TA in stromal cells and problems with the specificity of the currently available anti-hTERT antibodies that are suitable for IHC/IF make this task difficult. Interestingly, in a recent study, there were rare telomerase-expressing cells in murine endometrial stroma that may represent SPC cells (Deane et al., 2016). However, due to the fact that telomerase is expressed constitutively in many mouse tissues unlike in humans, it is difficult to evaluate the significance/relevance of this finding in the context of the human endometrium.

The only characterized human endometrial epithelial cell subpopulation (cells that express surface marker SSEA-1, nuclear SOX9 and nuclear β-catenin) that exhibits progenitor properties in vitro, also showed high TA and longer TL compared with their more differentiated epithelial cell counterparts (Valentijn et al., 2013; Gargett et al., 2016). Importantly, these cells with high TA were able to produce endometrial gland-like structures in 3D in vitro culture and when confronted with a 2D environment they were able to produce a mono-layer, functionally akin to the re-epithelialization of the denuded endometrial surface after shedding of the functionalis (Valentijn et al., 2015). Finally, in a study employing immunofluorescence microscopy, the potential stem cell marker Mushashi1 also co-localized with the telomerase protein hTERT in the endometrial epithelium (Gotte et al., 2008). However, Mushashi1 expressing cells have not been shown yet to have stem cell characteristics in functional studies.

Taken together, the above data suggest that endometrial SPC cells (basalis SSEA1+ epithelial cells and possibly stromal SPCs) have high TA (Valentijn et al., 2015). The exact function of telomerase in the epithelial and in stromal SPCs is not fully understood yet. Similar to the intestine, epidermis and other epithelial tissues and organs, endometrium may also have multiple, heterogeneous epithelial stem cell populations with or without a functional hierarchy (Schepers et al., 2011; Goodell et al., 2015; Pirvulet 2015) and they may have corresponding differential telomerase activation states. Active, more differentiated progenitor cells involved in normal physiological regeneration might have higher TA, while the true, dormant/quiescent stem cell population may not express any or very low levels of TA until they are activated. The quiescent stem cell population may have low TA during normal physiological regeneration of endometrium and only show high TA if challenged by extensive tissue disruption or when progenitors are compromised, such as after iatrogenic endometrial ablation (Hyama and Hiyama 2007; Biswas et al., 2015). Further studies on telomere biology and telomerase function in the endometrial stem cell population are required in order to elucidate altered pathways relevant to endometrial proliferative diseases. Since stem cells are hypothesized to harbour defects specific to chronic endometrial pathologies (Figueira et al., 2011; Gargett et al., 2014; Sourial et al., 2014), treatment strategies directed towards them may prove to be curative.

**Role of telomerase in pathological conditions of the endometrium**

**Endometriosis**

Endometriosis is a common chronic inflammatory disease, defined by the existence of endometrial-like stroma and epithelial tissue in ectopic sites, outside the uterine cavity. Since endometrial tissue is intensely responsive to ovarian hormones, the main stimulus for the growth of ectopic endometriotic lesions is estrogen (Sourial et al., 2014; Hapangama et al., 2015; Hapangama and Bulmer 2016; Kamal et al., 2016) and progesterone resistance has been proposed as a fundamental feature of ectopic endometriotic lesions (Bulun et al., 2006). The endometrium of women with endometriosis has been shown to be different to that of healthy fertile women (Hapangama et al., 2012; Mathew et al., 2016).

There is a large body of evidence demonstrating that high TA, expression of hTERT and protein levels associated with longer mean endometrial TLs are features of the eutopic secretory endometrium of women with endometriosis (Kim et al., 2007; Hapangama et al., 2008; Hapangama et al., 2009; Hapangama et al., 2010; Valentijn et al., 2013; Mafra et al., 2014; Valentijn et al., 2015). These changes have been proposed to contribute to the functional endometrial abnormalities that result in the clinical manifestation of subfertility and propagate ectopic lesions. Considering the known pro-survival function of telomerase, the high TA in late-secretory endometrium of women with endometriosis (Hapangama et al., 2008) might be responsible for the survival of cells that are shed into the peritoneal cavity during retrograde menstruation (Hapangama et al., 2010). The preferential survival of these cells and their enhanced replicative capacity due to high TA could facilitate implantation and/or establishment of ectopic lesions (Hapangama et al., 2010; Valentijn et al., 2015) (Fig. 4). This corresponds well with the finding of high TA and hTERT mRNA/protein levels in active peritoneal ectopic endometriotic lesions. In addition, ectopic epithelial cells display longer relative TL compared with eutopic epithelial cells from the same patient (Hapangama et al., 2008; Valentijn et al., 2015). This observation seems to be in agreement with the progesterone resistance described in the pathogenesis of endometriosis (Bulun et al., 2006; Sourial et al., 2014), where the development of ectopic endometriotic lesions may increase TA due to the failure of endogenous progesterone to inhibit telomerase at the ectopic site. We have already shown that dysregulation of telomerase is an important early change in endometriotic cells since high TA was required for the early establishment of ectopic lesions in a baboon model of induced endometriosis (Hapangama et al., 2010; Afshar et al., 2013) (Fig. 4).

Furthermore, in the baboon model, establishment of ectopic lesions was associated with induction of high TA and TERT expression in the eutopic endometrium (Hapangama et al., 2010). Interestingly, the initial induction of endometriosis was associated with activation of epidermal growth factor (EGF) signalling in the eutopic endometrium of the baboon model (Afshar et al., 2013) and EGF signalling was associated with up-regulation of TA in normal ovarian surface epithelial cells (Bermudez et al., 2008). A similar scenario might be happening in the eutopic endometrium in the baboon model. Eutopic endometrial cells with high TA can subsequently initiate more ectopic lesions after retrograde menstruation contributing to a self-propagation cycle of the disease (Hapangama et al., 2010) (Fig. 4). Ovarian endometriotic
Epithelial cells were successfully immortalized by combinatorial transfection of human cyclin D1, cdk4 and hTERT genes, whereas the introduction of hTERT alone, or together with cdk4, was insufficient for immortalization of these cells (Bono et al., 2012). Therefore, telomerase alone may not be sufficient for the apparent survival advantage displayed by the endometriotic cells (Fig. 4).

SPCs are suggested to play a key role in the pathogenesis of endometriosis (Figueira et al., 2011; Gargett et al., 2014; Sourial et al., 2014). Intriguingly, the epithelial cells of ectopic lesions show phenotypic similarities with SSEA1 expressing basalis epithelial cells (Valentijn et al., 2013; Valentijn et al., 2015). Recent data also suggests that the tumour suppressor protein ARID1A might negatively regulate hTERT transcription and TA. Induction of ARID1A repressed transcription of hTERT via binding to a regulatory element on the hTERT promoter, and promoted a repressive histone mode via occupying SIN3A and H3K9me3 (Rahmanto et al., 2016). ARID1A is a member of the SWI/SNF chromatin remodelling complex, and is reported to be frequently mutated in two epithelial ovarian carcinoma subtypes: ovarian clear cell carcinomas and endometrioid ovarian carcinomas (Samartzis et al., 2013; Grandi et al., 2015). These cancers have been molecularly and epidemiologically linked to endometriosis with approximately 20% of benign ovarian endometriosis lesions having a loss of BAF250a (encoded by ARID1A) expression (Xiao et al., 2012). Therefore, it is conceivable that hTERT expression may be potentially involved in carcinogenesis associated with the loss of ARID1A. However, the seemingly vigorous endometrial regulation of telomerase via ovarian hormones and the fact that TA levels are high and constitutively expressed in proliferating endometrial epithelial cells may be responsible for the apparently rare incidence of such transformation (Zafrakas et al., 2014; Ness et al., 2015). TL in peripheral lymphocytes of women with endometriosis compared to healthy controls did not differ in our studies (Hapangama et al., 2008; Hapangama et al., 2009) while others have reported longer telomeres in PBMCs from women with endometriosis (Draceljer et al., 2014). The reason for this observed difference could be attributed to the influence of different demographic features known to affect PMBC TL such as age, BMI and ethnicity. These factors were not accounted for and significantly differ between the two patient groups in the latter study and may account for the different TLs observed rather than endometriosis. Our studies controlled for these demographical parameters as well as the menstrual cycle phase and did not show an endometriosis associated significant difference in PMBC TLs (Hapangama et al., 2008; Hapangama et al., 2009).

Endometriosis shares some of the typical features of increased synthesis of pro-inflammatory cytokines and the imbalance between pro-inflammatory and anti-inflammatory cytokines with other chronic inflammatory diseases (Fig. 4, Souriel et al., 2014). Interestingly, telomere attrition and decreased TA have been associated with many chronic inflammatory diseases (Zhang et al., 2016). Although opposite changes in endometrial telomere regulation and involvement of
high TA in endometriosis have been confirmed by various studies to date (Kim et al., 2007; Hapangama et al., 2008, 2009, 2010; Valentijn et al., 2013; Mafra et al., 2014; Valentijn et al., 2015), this knowledge is yet to be translated into a therapeutic solution. Since endometriosis is postulated to be a progesterone resistant condition (Bulun et al., 2006) and since telomerase inhibition is a downstream effecter of progesterone (Valentijn et al., 2015), the option of telomerase inhibition must be further explored as an attractive, non-hormonal treatment for endometriosis.

**Endometrial polyps**

Endometrial polyps are defined as abnormal outgrowth of hyperplastic endometrial tissue consisting of a monoclonal overgrowth of endometrial stromal cells with inclusion of a non-neoplastic glandular component (Hapangama and Bulmer, 2016). Endometrial stimulation by estrogen is postulated as the main driving force for endometrial polyp formation and this is supported by the observation that the use of tamoxifen, which acts as an ER agonist on the endometrium, increases the risk of developing endometrial polyps. Lower levels of hTERT protein in endometrial polyps have been reported compared with normal endometrium in the proliferative phase (Hu and Yuan, 2011). CD146 expressing mesenchymal SPCs derived from endometrial polyps did not express any hTERT (Ding et al., 2011). Jointly these studies suggest that benign endometrial polyps with increased stromal growth have low telomerase and that TA is less likely to play an essential role in them. However, telomerase biology in polyps with epithelial hyperplasia/ataxia remains to be explored in future studies.

**Reproductive failure**

Embryo implantation occurs during the window of implantation in the mid-secretory phase. The mid-secretory phase is defined by the dominant action of progesterone with maximum cell differentiation in an environment where endometrial glandular proliferation indices are at their nadir. This period is also associated with the lowest endometrial inactivity of progesterone with maximum cell differentiation in an endometrial epithelium of fertile women has been proposed as a necessary process in unsuccessful cases implies a difference in telomerase regulation.

So far, the underlying mechanisms for the differential (dys)regulation are not understood. In a further case controlled study, infertile women with deep infiltrating endometriosis also had high endometrial telomerase expression further suggesting a detrimental effect of high levels of TA on conception/embryo implantation (Mafra et al., 2014). Progesterone is commonly employed to treat women with a variety of reproductive failures, from infertility, to luteal phase defects to recurrent miscarriages, yet the available evidence on the effectiveness of this therapy is inconclusive (Coomarasamy et al., 2016). Most of these conditions are multifactorial and the lesions from women included in clinical trials therefore are heterogeneous. This prevents elucidation of the true effectiveness of progesterone treatment in subgroups of women with an apparently similar clinical manifestation. Further examination of downstream effectors of progesterone treatment, such as telomerase, may enable the stratification of women in the future in order to identify those who may benefit from the administration of the hormone.

**Polycystic Ovarian Syndrome**

PCOS is a common gynaecological condition defined by the clinical manifestations of hormonal aberrations of hyperandrogenism and insulin resistance (Clark et al., 2014; Lizneva et al., 2016). It is often associated with anovulation and a subsequently increased risk of EH with risk of progression to EC (Hapangama et al., 2015; Hapangama and Bulmer, 2016; Kamal et al., 2016). A genome-wide association study in Korean women with PCOS has identified susceptibility loci for polycystic ovarian syndrome (PCOS) (Lee et al., 2015). The authors reported the strongest signal to be located upstream of KDH domain containing, RNA binding, signal transduction associated 3 (KHDRBS3). KHDRBS3 was found to regulate TA in colon cancer cells (Zhang et al., 2006). With this evidence, the authors concluded that telomerase may be an important driving force in developing PCOS and related phenotypes (Lee et al., 2015). Considering the suggested role that unopposed estrogen and excessive androgens have on TA (Boggess et al., 2006; Nourbakhsh et al., 2010) and cellular proliferation in various carcinoma cells including EC cells (Chao et al., 2013; Dumesic and Lobo, 2013; Plaza-Parrochia et al., 2014; Hapangama and Bulmer, 2016; Kamal et al., 2016), studies examining the involvement of telomerase in the endometrium of women with PCOS may unravel novel avenues for therapeutic manipulation.

**Malignant conditions of the endometrium**

Constitutively high levels of hTERT expression and TA have been identified in over 90% of human cancers including hepatocellular carcinoma, colorectal cancer and EC (Lehner et al., 2002; Saini et al., 2009; Bertorelle et al., 2014). High levels of TA in tumour cells contribute to increased cell proliferation, cellular immortality, carcinogenesis and cancer progression, while the activity of telomerase and hTERT expression are usually suppressed in most human somatic tissues (Cong et al., 1999). The involvement of telomerase in cellular immortality is further highlighted by the fact that most cell lines, including the first ever ‘immortal cell line’, cervical HeLa cells express very high levels of TA (Pandita et al., 1997). The involvement and activation of telomerase and telomere maintenance during tumorigenesis has been intensely studied over the years. In HeLa cells, as in many cervical cancers, the mechanism of telomerase activation is regulated by specific proteins from human papillomavirus Types 16.
and 18 (Kyo et al., 1996; Sakamoto et al., 2000; James et al., 2006).
Other cancers that do not involve viral infection in the pathogenesis also demonstrate high TA but the exact mechanism of their telomere activation is not fully understood. Telomerase suppressing mechanisms that are downstream of hTERT transcription and mRNA splicing are present in rapidly proliferating embryonic tissue (Ulener et al., 2000), but these are lost during neoplastic transformation (Ulener et al., 2000). Therefore, the tight physiological mechanisms of telomerase regulation do not exist in cancer cells where TA levels remain constitutively high.

There are reports of shorter or longer TLs in different cancer cells compared with their benign counterparts. Short TLs in cancer cells may result from excessive cell proliferation prior to telomere stabilization (with TA or an alternative telomere maintenance mechanism (ALT)). Telomere attrition can result in genomic instability which can subsequently initiate carcinogenesis. Absolute TL is therefore not relevant for cancer cells as long as telomeres are sufficiently maintained in a capped state in order to supply the cells with an indefinite proliferation capacity. There are at least four activating mutations reported in TERT, POT1, TPPI and TERF2IP (RAP1) genes of the telomerase and telomere complexes which can result in longer TLs while several other telomere and telomerase associated gene mutations (including repressor mutations in POT1 and activating mutations of TRF1/2) result in short TL (reviewed in Mengual Gomez et al., 2016). Since either lengthening or shortening of telomeres can result in abnormal cell proliferation or genomic instability, they can be implicated in carcinogenesis (reviewed inHolyz et al., 2013; Mengual Gomez et al., 2016; Shay, 2016). In this review, we examine the available evidence for specific aberrations in telomeres/telomerase in the premalignant and malignant conditions of the endometrium.

Endometrial hyperplasia
EH is characterized by irregular proliferation of endometrial glands that may precede or co-exist with endometrial carcinoma (Hapangama and Bulmer 2016; Kamal et al., 2016). Hyperplastic glands show extremely high proliferative indices in comparison to either stromal cells or normal glands during the proliferative phase (Dallenbach-Hellweg and Dallenbach, 2010). EH is almost always due to exposure to high estrogen levels and typically accompanied by a chronic insufficiency of progesterone. Classical causes therefore include corpus luteum insufficiency/anovulatory cycles, PCOS, obesity with metabolic syndrome (extra-ovarian aromatization of androgens) and inappropriate postmenopausal hormone therapy (tamoxifen, insufficient dosage of progestagens) (Hapangama and Bulmer 2016; Kamal et al., 2016).

High TA levels have been detected in EH, including the simple, complex and complex with atypia subtypes (Shroyer et al., 1997). It was also suggested that TA could be a useful diagnostic tool to screen postmenopausal women with endometrial premalignant and malignant lesions (Maida et al., 2002). However, there is a considerable proportion of EH samples included in these studies that lack TA (Shroyer et al., 1997) and the absence of detectable TA did not have a specific negative predictive value (Shroyer et al., 1997). Considering the method used to sample the endometrium (for example, an outpatient endometrial biopsy typically samples approximately 4% of the uterine cavity), it is unlikely that a small area of EH is reliably sampled and detected with this approach. Furthermore, since TA is a feature of normal proliferating endometrial cells, including simple hyperplasia, the level of TA is unlikely to be a sufficient discriminator to detect malignant or premalignant conditions of the human endometrium.

Endometrial cancer
Most previous research on endometrial telomerase had been focussed on EC that is associated with high TA. EC is the commonest gynaecological malignancy and is an estrogen driven disease (Kamal et al., 2016). The risk factors for EC include advanced age, obesity and exposure to unopposed estrogen (or progesterone deficiency). It is of interest that despite the high estrogenic milieu associated with the premenopausal period where most vigorous proliferative and regenerative activity takes place in the endometrium, carcinogenesis commonly occurs in the relatively quiescent, hypo-estrogenic postmenopausal period (Hapangama and Bulmer 2016; Kamal et al., 2016).

Very low but detectable TA levels are reported in the postmenopausal endometrium (Tanaka et al., 1998). Estrogen induces telomerase in a dose dependent manner (Kyo et al., 1999). Various risk factors (such as obesity) for EC will marginally increase the weak estrogen (estrone) levels in the postmenopausal endometrium (reviewed in Kamal et al., 2016). The intermittent and low levels of estrogen associated with these conditions (Kamal et al., 2016) may be sufficient for inducing low TA levels to initiate epithelial proliferation in the postmenopausal endometrial cells. Furthermore, both obesity and ageing are chronic inflammatory conditions that are associated with oxidative stress, thus accelerating telomere shortening in proliferating cells (von Zglinicki et al., 1995; von Zglinicki 2000; Kamal et al., 2016). Therefore the most likely mechanism to explain EC associated telomere aberrations is the insufficient TA/short-TL/damaged telomeres theory. We propose that the hypo-estrogenic hormonal maelstrom in high risk postmenopausal endometrium may be associated with a potentially deficient amount of TA that is insufficient to maintain the short endometrial TLs and their integrity during cell division. Therefore, the ongoing proliferation in postmenopausal epithelial cells with short TLs may render them vulnerable to subsequent genetic instability and carcinogenic transformation similar to various other cancer types (Greider and Blackburn 1996; Bertorelle et al., 2014; Meena et al., 2015). In line with the above hypothesis, short TLs have been reported in most sporadic ECs.

Lynch syndrome is an autosomal dominant condition characterized by germ line mutation in DNA mismatch repair genes resulting in increased risk of developing a variety of cancers including EC. Long telomeres are associated with familial cancer syndromes such as familial melanoma (Akbay et al., 2008; Horn et al., 2013). However, short TLs and hTERT gene polymorphisms predict initiation of EC in Lynch patients (Segui et al., 2013).

High TA, hTERT and hTERC expression as well as high hTERT protein levels have been described by many groups in ECs (Kyo et al., 1996; Saito et al., 1997; Ebina et al., 1999; Maida et al., 2002). Some preliminary data in a very small patient population also suggests that hTERT mRNA in PBMCs can be used to diagnose early micro-metastases of EC (Liang et al., 2016). This observation needs further validation in an appropriately powered study. Frequent PTEN mutations and P53 loss known to occur in ECs could be associated with telomerase up-regulation but the exact mechanism through which these different events interact is not yet clear (Zhou et al., 2006; Akbay et al., 2013). Further recent work also showed hTERT to be involved in EMT, cell motility and metabolism, which are processes associated
with cancer metastasis (reviewed in Saretzki, 2014). In a mouse model, simultaneous deletion of p53 and POT1 resulted in precursor lesions of endometrial epithelium and induced ECs with non-endometroid, Type II phenotype, suggesting that telomeric instability has a critical role to play in Type II ECs (Akbay et al., 2013). TERT promoter mutations seem to be rare in ECs (Wu et al., 2014), except for the clear cell (Type II EC) subtype (Huang et al., 2015). Activating TERT promoter mutations are a feature of cancers derived from tissues with low relative cellular turnover such as the brain, therefore such mutations are not expected in the highly regenerative endometrial epithelium (Killela et al., 2013; Shay 2016).

There are limited reports suggesting that the ALT pathway may be active in some endometrial carcino-sarcomas or uterine sarcomas (Lee et al., 2012), but its prevalence in endometroid ECs appears to be low (Heaphy et al., 2011). Further studies are required to dissect out the exact function high TA plays in ECs and how telomerase regulation in the postmenopausal endometrium may induce carcinogenesis, particularly, in the context where a high risk hormonal environment is present. In the light of many reported alterations in the components of the telomere complex, telomerase holoenzyme and their associated proteins in cancer (Greider and Blackburn, 1996; Shay, 2016), in addition to the complex interactions of these changes with numerous tumour suppressor proteins and oncogenes, more detailed studies are required to fully understand the role of alterations in the telomere maintenance pathways particular to all subtypes of EC.

Future directions and wider implications of interventions into telomerase biology

hTERT has been reported to be up-regulated by various pharmacological and metabolic agents such as angiotensin-converting enzyme inhibitors (Donnini et al., 2010), essential fatty acids (Das 2008), beta blockers (Wang et al., 2011) and calcium channel blockers (Hayashi et al., 2014) in a variety of tissue types. Telomerase inhibition has been reported as an off-target effect of various chemotherapeutic agents, however this it could be a secondary effect to apoptosis or senescence induction (Saretzki, 2010). Imetelstat is the only clinically applicable synthetic telomerase inhibitor, which is a lipid based conjugate of the oligonucleotide GRN163 that binds with high affinity to the hTERC component of telomerase (Roth et al., 2010; Salloum et al., 2016). Imetelstat had shown promising preclinical telomerase inhibitory activity across a wide range of cancer types (Roth et al., 2010). However, despite high expectations, all reported data on its clinical effectiveness in at least six different clinical cancer trials have been disappointing (Salloum et al., 2016), with treatment resulting in significant side effects such as myelo-suppression. Findings from a recent small clinical pilot study demonstrate a possible specific beneficial effect of Imetelstat in clinical outcomes in myelo-proliferative conditions that needs to be further confirmed in larger trials (Baerlocher et al., 2015). The side effect profile of this drug, however, precludes its use in benign endometrial conditions. The examination of different compounds with off-target inhibitory effects on TA and a more favourable side effect profile is required before clinical application in endometrial disease.

Our understanding of telomerase and telomere biology is rapidly expanding with many groups working towards developing this exciting field, as telomeres and telomerase pertain to a diverse range of cellular functions and processes. In addition, telomerase regulation and function in the human endometrium appears to be unique. The majority of treatments we have available at present for common benign and chronic diseases of the endometrium such as heavy menstrual bleeding, infertility, polyps, endometriosis and EH are directed towards terminally differentiated endometrial cells of the endometrial functionalis. The functionalis is shed each month with menstruation, thus requiring continuous therapy for each new functionalis layer that is regenerated. Most currently available therapeutic options therefore include ovarian or other hormonal analogues to manipulate the ovarian cycle resulting in considerable side effects. Since telomerase appears to be involved in almost all endometrial pathologies, it provides a distinctive route to formulate possible curative (involving stem cells), non-hormonal therapies to treat them. Furthermore, our current understanding of telomere and telomerase biology in ECs is far from comprehensive and the fact that high TA is present in normal proliferating endometrial cells makes it difficult to extrapolate the data from other cancers to ECs. TA in normal endometrium has strict physiological regulation, mainly by hormones. During tumourigenesis this regulation becomes dysfunctional, supplying cells with a constitutively high amount of TA, conferring a selective advantage and a high proliferative capacity. In addition, telomerase and telomere biology of the endometrial cells may be modified by the cellular interactions in a new environment (e.g. endometriotic lesions growing in the peritoneal cavity). A better understanding of these processes may facilitate formulating new interventions. Further studies in this field for both benign and malignant diseases of the endometrium should therefore focus on understanding the precise regulation mechanisms for telomerase which could potentially reveal novel targets for new treatment strategies based on telomerase in endometrial disease.

Conclusion

We are only beginning to understand the central role of telomeres and telomerase in the biology of the endometrium. Since telomerase is pivotal to endometrial regeneration, further studies elucidating the involvement of telomere and telomerase associated proteins and their regulation in normal endometrial regeneration, and in endometrial pathologies, will help in developing novel treatment strategies that are not only hormonal but also potentially curative.

Acknowledgements

The authors are grateful to Mr Anthony Valentijn (isolation of endometrial cells for the data included in Fig. 3), Dr Nicola Tempest (qFISH in Fig. 3), Dr David Mathew and Mr Druvi Edirisinghe (language editing) for their assistance with study and manuscript preparation.

Authors’ roles

D.H. conceived the manuscript design and prepared the first draft. G. S. revised the manuscript critically for important intellectual content.
D.H./A.K. drafted the figures and tables. All authors reviewed and read the manuscript and approved the submitted final version.

**Funding**

We acknowledge the support by Wellbeing of Women project grant RG1073 (D.K.H./G.S.) and RG1487 (D.K.H./G.S.).

**Conflict of interest**

None declared.

**References**

Afshar Y, Hastings J, Roqueudo D, Jeong JW, Giudice LC, Fazleabas AT. Changes in eutopic endometrial gene expression during the progression of experimental endometriosis in the baboon, Papio anubis. *Biol Reprod* 2013;88:44.

Ahmed S, Passos JF, Birket MJ, Beckmann T, Brings S, Peters H, Birch-Machin MA, von Zglinicki T, Saretzki G. Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. *J Cell Sci* 2008;121:1046–1053.

Aix E, Gutierrez-Gutierrez O, Sanchez-Ferrer C, Aguado T, Flores I. Postnatal telomere dysfunction induces cardiomyocyte cell-cycle arrest through p21 activation. *J Cell Biol* 2016;213:571–583.

Akbaa E, Contreras CM, Perera SA, Sullivan JP, Broaddu RR, Schorge JG, Ashfaq R, Saboorian H, Wong KK, Castrillon DH. Differential roles of telomere attrition in type I and II endometrial carcinogenesis. *Am J Pathol* 2008;173:536–544.

Akbaa E, Pena CG, Ruder D, Michel JA, Nakada Y, Pataski S, Muliansi AT, Chang S, Castrillon DH. Cooperation between p53 and the telomere-protecting shelterin component Pot1a in endometrial carcinogenesis. *Oncogene* 2013;32:2211–2219.

Ainclair SC, Urail B, Tergaonkar V. Reactivation of telomerase in cancer. *Cell Mol Life Sci* 2016;73:1659–1670.

Akiyama M, Hideshima T, Hayashi T, Tai YT, Mitsiades CS, Mitsiades N, Chauhan D, Akbay EA, Contreras CM, Perera SA, Sullivan JP, Broaddus RR, Schorge JO, Ashfaq A, Angrisani A, Vicidomini R, Turano M, Furia M. Human dyskerin: beyond telomeres. *Aging Cell* 2015;14:913–921.

Akeroyd M, Bunting B, Snyder DS. Telomerase inhibitor inmetast in essential thrombocythemia and myelofibrosis. *N Engl J Med* 2015;373:2570.

Barbier CS, Becker KA, Troester MA, Kaufman DG. Expression of exogenous human telomerase in cultures of endometrial stromal cells does not alter their hormone responsiveness. *Biol Reprod* 2005;73:106–114.

Barrett EL, Richardson DS. Sex differences in telomeres and lifespan. *Aging Cell* 2011;10:913–921.

Batista LF. Telomere biology in stem cells and reprogramming. *Prog Mol Biol Transl Sci* 2014;125:67–88.

Baumann P, Price C. Pot1 and telomere maintenance. *FEBS Lett* 2010;584:3779–3784.

Bekaert S, De Meyer T, Van Oostveldt P. Telomere attrition as ageing biomarker. *Anticancer Res* 2005;25:3011–3021.

Benetos A, Kark JD, Susser E, Kimura M, Sinnreich R, Chen W, Steenstrup T, Christensen K, Herbig U, von Bornemann Hjelmborg J et al. Smoking and telomere length in Danish twin sisters: a longitudinal study. *Int J Cancer* 2011;128:2656–2663.

Benetos A, Kimura M, Labat C, Buchoff GM, Huber S, Labat L, Liu X, Aviv A. A model of canine leukocyte telomere dynamics. *Biol Reprod* 2010;83:1213–1217.

Beyer AM, Fiedl RJ, Durand MJ, Riedel M, Ait-Aissa K, Green P, Hockenberry JC, Morgan RG, Donato AJ, Peleg R et al. Critical role for telomerase in the mechanism of flow-mediated dilation in the human microcirculation. *Circ Res* 2016;118:856–866.

Biswas S, Davis H, Irshad S, Sandberg T, Worthley D, Leedham S. Microenvironmental control of stem cell fate in intestinal homeostasis and disease. *J Pathol* 2015;237:135–145.

Blackburn EH. Telomeres: do the ends justify the means? *Cell* 1984;37:7–8.

Blackburn EH. Walking the walk from genes through telomere maintenance to cancer risk. *Cancer Prev Res (Phila)* 2011;4:473–475.

Blackburn EH, Chan S, Chang J, Fulton TB, Krauskopf A, McEacharn M, Prescott J, Roy J, Smith C, Wang H. Molecular manifestations and molecular determinants of telomere capping. *Cold Spring Harb Symp Quant Biol* 2000;65:253–263.

Blackburn EH, Collins K. Telomerase: an RNP enzyme synthesizes DNA. *Cold Spring Harb Perspect Biol* 2011;3(5). pii: a003558.

Blackburn EH, Eipel ES, Lin J. Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. *Science* 2015;350:1193–1198.

Blackburn EH, Gall JC. A tandemly repeated sequence at the termini of the extra-chromosomal ribosomal RNA genes in Tetrahymena. *J Mol Biol* 1978;120:33–53.

Bodvarsdottir SK, Steinarsdottir M, Bjarnason H, Eyfjord JE. Dysfunctional telomeres in human BRCA2 mutated breast tumors and cell lines. *Mutat Res* 2012;729:90–99.

Bogess JF, Zhou CX, Bae-Jump VL, Gehrig PA, Whang YE. Estrogen-receptor-dependent regulation of telomerase activity in human endometrial cancer cell lines. *Gynecol Oncol* 2006;103:417–424.

Bojovic B, Booth RE, Jin Y, Zhou X,Crowe DW. Alternative lengthening of telomeres in cancer stem cells in vivo. *Oncogene* 2015;34:611–620.

Bonaz G, Klapper W, Barthe A, Heidorn K, Jonat W, Krupp G, Parwaresch R. Analysis of telomerase expression and proliferative activity in the different layers of human endometrium. *Biochim Biophys Res Commun* 1999;253:214–217.

Bono Y, Kyo S, Takakura M, Maida Y, Mizumoto Y, Nakamura M, Nomura K, Kiyono T, Inoue M. Creation of immortalised epithelial cells from ovanian endometrioma. *BJ Cancer* 2012;106:1201–1215.

Bren T, Kallakury BV, Lawoye CV, Ambros RA, Muraca PJ, Malfetano JH, Ross JS. Telomerase activity in benign endometrium and endometrial carcinoma. *Cancer Res* 1997;57:2760–2764.

Bryant TM, Manusci L, Baczetti S, Namba M, Reddel RR. The telomere lengthening mechanism in telomerase-negative immortal human cells does not involve the telomerase RNA subunit. *Hum Mol Genet* 1997;6:921–926.

Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, Innes J, Lime Kim J. Progestosterone resistance in endometriosis: link to failure to metabolize estradiol. *Mol Cell Endocrinol* 2006;248:94–103.

Bunch K, Tinnemore D, Huff S, Hoffer ZS, Burney RO, Stallings JD. Expression patterns of telomerase receptor membrane components 1 and 2 in endometria from women with and without endometriosis. *Reprod Sci* 2014;21:190–197.

Calado RT, Dumitru B. Telomere dynamics in mice and humans. *Semin Hematol* 2013;50:165–174.

Calado RT, Yewdell WT, Wilkinson KL, Regal JA, Kajigaya S, Stratakis CA, Young NS. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. *Blood* 2009;114:2236–2243.

Cameiro MC, Henriques CM, Nabiás J, Ferreira T, Carvalho T, Ferreira MG. Short telomeres in key tissues initiate local and systemic aging in zebrafish. *PLoS Genet* 2016;12:e1005798.

Cawthon RM, Smith KR, O’Brien E, Svavatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 2003;361:393–395.

Cen J, Zhang H, Liu Y, Deng M, Tang S, Liu W, Zhang Z. Anti-aging effect of estrogen on telomerase activity in ovariectomised rats—animal model for menopause. *Gynecol Endocrinol* 2013;29:582–585.

Cerrudo CS, Mengual Gomez DL, Gomez DE, Ghiiringelli PD. Novel insights into the evolution and structural characterization of dyskerin using comprehensive bioinformatics analysis. *J Proteome Res* 2015;14:874–887.

Cervello I, Gil-Sanchis C, Mas A, Faus A, Sanz J, Mascaro F, Higuera G, Sanz MA, Pellicer A, Simon C. Bone marrow-derived cells from male donors do not contribute to the endometrial side population of the recipient. *PloS One* 2012;7:e30260.

Cervello I, Mas A, Gil-Sanchis C, Peris L, Faus A, Saunders PT, Critchley HO, Simon C. Reconstruction of endometrium from human endometrial side population cells. *PloS One* 2011;6:e21221.

Cesare AJ, Karledsen J. A three-state model of telomere control over human proliferative boundaries. *Curr Opin Cell Biol* 2012;24:731–738.
Cimino-Reale G, Pascale E, Battiloro E, Starace G, Verna R, D Chu TW, D Chronowska E. Regulation of telomerase activity in ovarian granulosa cells.

Chen LY, Zhang Y, Zhang Q, Li H, Luo Z, Fang H, Kim SH, Qin L, Yotnda P, Xu J Cha Y, Kwon SJ, Seol W, Park KS. Estrogen receptor-alpha mediates the effects of estrogen on telomerase activity in human mesenchymal stem cells. Mol Cells 2008;26:454–458.

Chan RW, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod 2004;70:1738–1750.

Chao A, Lin CY, Tsai CL, Huheu S, Lin YY, Lin CT, Chou HH, Wang TH, Lai CH, Wang HS. Estrogen stimulates the proliferation of human endometrial cancer cells by stabilizing nucleophosmin/823 (NPM/823). J Mol Med (Berl) 2013;91:249–259.

Chen LY, Zhang Y, Zhang Q, Li H, Luo Z, Fang H, Kim SH, Qin L, Yotnda P, Xu J et al. Mitochondrial localization of telomeric protein TIN2 links telomere regulation to metabolic control. Mol Cell 2012;47:839–850.

Chiappi J, Kim SH, Tesarollo L, Campisi J, Hodes RJ. Telomerase-associated protein TIN2 is essential for early embryonic development through a telomerase-independent pathway. Mol Cell Biol 2004;24:6631–6634.

Chirgadze A, Muniandy S, Saleh N. Combinatorial effect of genistein and female sex-steroids on uterine fluid volume and secretion rate and aquaporin (AQP)-1, 2, 5, and 7 expression in the uterus in rats. Environ Toxicol 2016; DOI: 10.1002/tox.22283 [In press].

Chronowski E. Regulation of telomerase activity in ovarian granulosa cells. Indian J Exp Biol 2012;50:595–601.

Chu TW, D’Souza Y, Autexier C. The insertion in fingers domain in human telomerase can mediate enzyme processivity and telomerase recruitment to telomeres in a TPPI-dependent manner. Mol Cell Biol 2016;36:210–222.

Chung J, Khadka P, Chung IK. Nuclear import of hTERT requires a bipartite nuclear localization signal and Akt-mediated phosphorylation. J Cell Sci 2012;125:2684–2697.

Cimino-Reale G, Pascale E, Battiloro E, Starace G, Verna R, D’Ambrosio E. The length of telomeric G-rich strand 3’ overhang measured by oligonucleotide ligation assay. Nucleic Acids Res 2001;29:E35.

Clark NM, Podolski AJ, Brooks ED, Chazen DR, Pierson RA, Lehota DC, Lujan ME. Prevalence of polycystic ovary syndrome phenotypes using updated criteria for polycystic ovarian morphology: an assessment of over 100 consecutive women self-reporting features of polycystic ovary syndrome. Metabolism 2013;62:1850–1857.

De Boeck G, Forsyth RG, Praet M, Hogendoorn PC. Telomere-associated protein TIN2 regulates cell senescence by inducing apoptosis, independently of telomere maintenance. Stem Cells 2014;32:776–784.

Draculcu CR, Oh C, Kim SH, Tessarollo L, Campisi J, Hodes RJ. Telomere-associated protein TIN2 is essential for early embryonic development through a telomerase-independent pathway. Mol Cell Biol 2004;24:6631–6634.

Chiang YJ, Kim SH, Tessarollo L, Campisi J, Hodes RJ. Telomere-associated protein TIN2 is essential for early embryonic development through a telomerase-independent pathway. Mol Cell Biol 2004;24:6631–6634.

Das UN. Can essential fatty acids reduce the burden of disease(s)? Lipids Health Dis 2008;7.

De Boeck G, Forsyth RG, Praet M, Hogendoorn PC. Telomere-associated protein TIN2: cross-talk between telomere maintenance and telomere-lengthening mechanisms. J Pathol 2009;217:327–344.

de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev 2005;19:2100–2110.

Deane JA, Ong YR, Cain JE, Jayasekara WS, Tiwari A, Carlone DL, Watkins DN, Breault DT, Gargett CE. The mouse endometrium contains epithelial, endothelial and leukocyte populations expressing the stem cell marker telomerase reverse transcriptase. Mol Hum Reprod 2016;22:272–284.

De Bufalo D, Rizzo A, Trisciuoglio D, Cardinali G, Torrisi MR, Zangemeister-Wittke U, Zupi G, Broccio A. Involvement of hTERT in apoptosis induced by interference with Bel-2 expression and function. Cell Death Differ 2005;12:149–148.

Ding DC, Chu TY, Chou SH, Liu HW. Enhanced differentiation and clonogenicity of human endometrial polyp stem cells. Differentiation 2011;81:172–180.

Dojusubroto MW, Choi YS, Lee HW, Rudolph KL. Telomeres and telomerase in aging, regeneration and cancer. Mol Cells 2003;15:164–175.

Donnini S, Terzuoli E, Ziche M, Morbidelli L. Sulphhydryl angiotsensin-converting enzyme inhibitor promotes endothelial cell survival through nitric-oxide synthase, fibroblast growth factor-2, and telomerase cross-talk. J Pharmacol Exp Ther 2010; 332:776–784.

Draculcu CR, Oh C, Kim SH, Tessarollo L, Campisi J, Hodes RJ. Telomere-associated protein TIN2 is essential for early embryonic development through a telomerase-independent pathway. Mol Cell Biol 2004;24:6631–6634.
Goodell MA, Nguyen H, Shroyer N. Somatic stem cell heterogeneity: diversity in the blood, skin and intestinal stem cell compartments. *Nat Rev Mol Cell Biol* 2015;16:299–309.

Gopalakrishnan S, Cheung NKM, Yip BWP, Au DWT. Medaka fish exhibits longevity gender gap, a natural drop in estrogen and telomere shortening during aging: a unique model for studying sex-dependent longevity. *Front Zool* 2013;10:78.

Gott M, Wolf M, Staebler A, Buchweitz O, Kelsch R, Schuring AN, Kiesel L. Increased expression of the adult stem cell marker Musashi-1 in endometriosis and endometrial carcinoma. *J Pathol* 2008;215:317–329.

Grandi G, Toss A, Cortesi L, Botticelli L, Volpe A, Cagnacci A. The association between endometriomas and ovarian cancer: preventive effect of inhibiting ovulation and menstruation during reproductive life. *Boned Res Int* 2015;2015:1–10.

Greider CW, Blackburn EH. Telomeres, telomerase and cancer. *Science* 1997;274:92–97.

Griffith JD, Comeau L, Rosenfeld S, Stansel RM, Bianchi A, Moss H, de Lange T. Mammalian telomeres end in a large duplex loop. *Cell* 1999;97:503–514.

Hapangama DK. Mifepristone: the multi-faceted anti-hormone. *J Drug Eval* 2003;1:149–175.

Hapangama DK, Bulmer JN. Pathophysiology of heavy menstrual bleeding. *Women Health (Lond)* 2016;12:13–

Hapangama DK, Kamal AM, Bulmer JN. Estrogen receptor beta: the guardian of reproductive life. *Am J Reprod Immunol* 2012;69:291–309.

Hapangama DK, Turner MA, Drury JA, Martin-Ruiz C, Von Zglinicki T, Farquharson Zglinicki. Endometriosis is associated with aberrant endometrial expression of the adult stem cell marker Musashi-1 in endometriosis and endometrial carcinoma. *J Pathol* 2008;215:317–329.

Hapangama DK, Turner MA, Drury JA, Cadiz GA, Drury JA, Martin-Ruiz C, Von Zglinicki T, Farquharson RG, Quenby S. Endometrial telomerase shows specific expression patterns in different types of reproductive failure. *Repam* 2008;18:416–424.

Hapangama DK, Turner MA, Drury JA, Quenby S, Crilly L, Martin-Ruiz C, von Zglinicki T. Sustained replication in endometrium of women with endometriosis occurs without evoking a DNA damage response. *Hum Reprod* 2009;24:687–696.

Hapangama DK, Turner MA, Drury JA, Quenby S, Saretzki G, Martin-Ruiz C, Von Zglinicki T. Androgen receptors are acquired by healthy postmenopausal epithelial cells: implications for the pathogenesis of endometriosis. *Hum Reprod* 2012;27:394–407.

Hapangama DK, Turner MA, Drury JA, Heathcote L, Afshar Y, Mavrogianis PA, Hombach-Klonisch S, Kehlen A, Fowler PA, Huppertz B, Jugert JF, Bischoff G, Hoffmeyer K, Raggioli A, Rudloff S, Anton R, Hierholzer A, Del Valle I, Hein K, Holysz H, Lipinska N, Paszel-Jaworska A, Rubis B. Telomerase as a useful target in cancer fighting-the breast cancer case. *Tumor Biol* 2013;34:1371–1380.

Horn-S, Fgl A, Rachakonda PS, Fischer C, Sucker A, Gatz A, Kadel S, Moll I, Nagore E, Hemminki K et al. TERT promoter mutations in familial and sporadic melanoma. *Science* 2013;339:959–961.

Hrdlickova R, Nehyba J, Buse HR Jr. Alternatively spliced telomerase reverse transcriptase variants lacking telomerase activity stimulate cell proliferation. *Mol Cell Biol* 2012;32:4283–4296.

Hu JG, Yuan R. The expression levels of stem cell markers importin13, c-kit, CD146, and telomerase are decreased in endometrial polyps. *Med Sci Monit* 2011;17:Br221–Br227.

Huang CH, Yang YC, Cheng WF, Chen CA, Lin MC, Kuo KT. Molecular alterations in endometrial and ovarian clear cell carcinomas: clinical impacts of telomerase reverse transcriptase promoter mutation. *Mod Pathol* 2015;28:303–311.

Huang Y, Liang P, Liu D, Huang J, Songyang Z. Telomere regulation in pluripotent stem cells. *Protein Cell* 2014;5:194–202.

Hug N, Lingner J. Telomere length homeostasis. *Chromosoma* 2006;115:413–425.

Jacob NK, Lescasre R, Linger BR, Price CM. Tetralymena POT1a regulates telomere length and prevents activation of a cell cycle checkpoint. *Mol Cell Biol* 2007;27:1592–1601.

James MA, Lee JH, Klingelhoft AJ. HPV16-E6 associated hTERT promoter acetylation is a6AP dependent, increased in later passage cells and enhanced by loss of p300. *Int J Cancer* 2006;119:1878–1885.

Kamal A, Tempest N, Parkes C, Alnahlah F, Makrydama S, Adishesh M. Hapangama DK. Hormones and endometrial carcinogenesis. *Hum Mol Biol Clin Invest* 2016;25:129–148.

Kamal AM, Bulmer JN, DeCruze SB, Stringfellow HF, Martin-Hirsch P, Hapangama DK. Androgen receptors are acquired by healthy postmenopausal epithelial cell types. *Hum Reprod* 2016;11:688–696.

Kearns G, Liu D, Rasmussen LJ. *Exonuclease I* and its versatile roles in DNA repair. *Cuir Rev Biochem Mol Biol* 2016;1:12.

Kibe T, Osawa GA, Keegan CE, de Lange T. Telomere protection by TPP1 is mediated by POT1a and POT1b. *Mol Cell Biol* 2010;30:1059–1066.

Killeja PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA Jr., Friedman AH, Friedman H, Gallia GL, Giovanna et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci USA* 2013;110:6021–6026.

Kim CM, Oh YJ, Cho SH, Chung DJ, Hwang JY, Park KH, Choi DJ, Choi YM, Lee BS. Increased telomerase activity and human telomerase reverse transcriptase mRNA expression in the endometrium of patients with endometriosis. *Hum Reprod* 2007;22:843–849.

Kimura A, Ohmichi M, Kawagoe J, Kyo S, Mabuchi S, Takahashi T, Ohshima C, Arimoto-Ishida E, Nishio Y, Incoue M et al. Induction of hTERT expression and phosphorylation by estrogen via Akt cascade in human ovarian cancer cell lines. *Oncoogene* 2004;23:4505–4515.

King AE, Collins F, Klessant K, Sallenave JM, Critchley HO, Saunders PT. An additive interaction between the NFkappaB and estrogen receptor signaling pathways in human endometrial epithelial cells. *Hum Reprod* 2010;25:510–518.

Kipling D, Cooke HJJ. Hypervariable ultra-long telomeres in mice. *Nature* 1990;347:400–402.

Kiss T, Fayet-Lebaron E, Jady BE, Box H/AACA small ribonucleoproteins. *Mol Cell* 2010;37:597–606.

Kyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelhoft AJ. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* 1998;396:84–88.

Knight SW, Heiss NS, Vulliamy TJ, Greeshner S, Stavrides G, Pai GS, Lestringant G, Knight SW, Heiss NS, Vulliamy TJ, Greeshner S, Stavrides G, Pai GS, Lestringant G, Pai GS.

Korch C, Spillman MA, Jackson TA, Jacobsen BM, Murphy DK, Lessey BA, Jordan VC, Bradford AP. DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination. *Gynecol Oncol* 2012;127:241–246.

Krokus G, Mar G, Lockwood C. The immortalization of human endometrial cells. *Methods Mol Med* 2006;121:79–83.

Kruuv AHN. Polyepitope components of telomere nucleoprotein complex. *Biochemistry (Mosc)* 2004;69:117–129.

Kurita T, Young P, Brody JR, Lydon JP, O’Malley BW, Cunha GR. Stromal progesterone receptors mediate the inhibitory effects of progestrone on estrogen-induced uterine epithelial cell deoxyribonucleic acid synthesis. *Endocrinology* 1998;139:4708–4713.
Kusama K, Yoshih M, Tamura K, Daikoku T, Takarada T, Tachikawa E. Possible roles of the CAMP-mediatedors EPAC and RAP1 in decidualization of rat uterus. Reproduction 2014;147:897–906.

Kyo S, Kanaya T, Ishikawa H, Ueno H, Inoue M. Telomerase activity in gynecological tumors. Clin Cancer Res 1996;2:2023–2028.

Kyo S, Nakamura M, Kiyono T, Mada Y, Kanaya T, Tanaka M, Yatabe N, Inoue M. Successful immortalization of endometrial glandular cells with normal structural and functional characteristics. Am J Pathol 2003;163:2259–2269.

Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, Orimo A, Inoue M. Estrogen activates telomerase. Cancer Res 1999;59:5917–5921.

Kyo S, Takakura M, Kohama T, Inoue M. Telomerase activity in human endometrium. Cancer Res 1997;57:610–614.

Lange CA, Richer JK, Horwitz KB. Hypothesis: progesterone primes breast cancer cells for cross-talk with proliferative or antiproliferative signals. Mol Endocrinol 1999;13:829–836.

Lebeau J, Fouchet P, Ory K, Chevillard S. Down-regulation of telomerase activity after progesterone treatment of human breast cancer cells: essential role of the cell cycle status. Antisense Res 2002;22:2161–2166.

Lee H, Oh JY, Sung YA, Chung H, Kim HL, Kim GS, Cho YS, Kim JT. Genomewide association study identified new susceptibility loci for polycystic ovary syndrome. Hum Reprod 2015;30:723–731.

Lee SS, Bohrson C, Pike AM, Wheelan SJ, Greider CW. ATM kinase is required for telomere elongation in mouse and human cells. Cell Rep 2015;13:1632–1637.

Lee YK, Park NH, Lee H. Prognostic value of alternative lengthening of telomeres. Cancer Cell 2009;22:434–441.

Lehner R, Enomoto T, McGregor JA, Shroyer AL, Haugen BR, Pugazhenthi U, Shroyer KR. Quantitative analysis of telomerase hTERT mRNA and telomerase activity in endometrioid adenocarcinoma and in normal endometrium. Gynecol Oncol 2002;84:120–125.

Leon-Blanco MM, Guerrero JM, Reiter RJ, Pozo D. RNA expression of human telomerase subunits TR and TERT is differentially affected by melatonin receptor agonists in the MCF-7 tumor cell line. Cancer Lett 2004;216:73–80.

Leri A, Malhotra A, Liew CC, Kajstura J, Anversa P. Telomerase activity in rat cardiac myocytes is age and gender dependent. J Mol Cardiol 2000;32:385–390.

Lewis KA, Tollefsbol TO. Regulation of the telomerase reverse transcriptase subunit on Wnt signaling in breast cancer cells. Mol Genet Metab 2007;91:85–92.

Li J, Yin G, Chen M, Wu A. Detection of hTERT mRNA in peripheral blood mononuclear cells in chronic myelomonocytic leukemia. Genes Dev 2005;19:1127.

Liu Z, Li Q, Li K, Chen L, Li W, Hou M, Liu T, Yang J, Lindvall C, Bjorkholm M, Mogensen UM, Rhodin J. Telomerase activity in endometrioid adenocarcinoma and in normal endometrium. J Cancer Res Clin Oncol 2012;138:971–977.

Leri A, Malhotra A, Liew CC, Kajstura J, Anversa P. Telomerase activity in normal mammalian somatic cells. Genes Dev 2013;27:18–23.

Mazudra H, Matsuzaki Y, Hirata E, Ono M, Nagashima T, Kajitani T, Arase T, Oda H, Uchida H, Asada H et al. Stem cell-like properties of the endometrial side population: implication in endometrial regeneration. Plur One 2010;5:e10387.

Mathew D, Drury JA, Valentin AJ, Vasieva O, Hapangama DK. In silico, in vitro and in vivo analysis identifies a potential role for steroid hormone regulation of FOXD3 in endometriosis-associated genes. Hum Reprod 2016;31:345–354.

Meena J, Rudolph KL, Gunes C. Telomere dysfunction, chromosomal instability and cancer. Recent Results Cancer Res 2015;200:61–79.

Mengual Gomez DL, Armando RG, Cerrudo CS, Ghiringhelli PD, Gomez DE. Telomerase as a cancer target. Development of new molecules. Curr Top Med Chem 2016;16:2432–2440.

Mikkelsen VM, Gamaiey LA. Telomere shortening is a sole mechanism of aging in mammals. Curr Aging Sci 2012;5:203–208.

Missi S, Nanni S, Fontemaggi G, Cong YS, Wen J, Hirve HV, Piaggio G,Sacchi A, Pontecorvi A, Bachetti S et al. Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelial cells. Mol Cell Biol 2000;20:3764–3771.

Moody AD, Cech TR. Low abundance of telomerase in yeast: implications for telomerase h RNAs. Science 2000;286:712–717.

Muezzinier A, Zaineddin AK, Brenner H. A systematic review of leukocyte telomere length and age in adults. Ageing Res Rev 2013;12:509–519.

Nakamura TM, Morin GB, Chapman KB, Weinreich SL, Andrews WH, Lingner J, Harley CB, Cech TR. Telomerase catalytic subunit homologs from fission yeast and human. Science 1997;277:955–959.

Nandakumar J, Cech TR. Finding the end: recruitment of telomerase to telomeres. Nat Rev Mol Cell Biol 2013;14:69–82.

Nanni S, Narducci M, Della Pietra L, Moretti F, Grasselli A, De Parli C, Sacchi A, Pontecorvi A, Farsetti A. Signaling through estrogen receptors modulates telomerase activity in human prostate cancer. J Clin Invest 2002;110:219–227.

Ness R, Pearce C, Stram D, Berchuck A, Pike M, Pharoah P. Lifetime risk of ovarian cancer based on endometriosis and other risk factors. Int J Gynecol Cancer 2015;25:50–51.

Neumna AA, Watson CM, Noble JR, Picketh HA, Tam PP, Reddel RR. Alternative lengthening of telomeres in normal mammalian somatic cells. Genes Dev 2013;27:18–23.

Nourbakhsh M, Golestanii A, Zahrai M, Mardarrehi MH, Malekpour Z, Karami Tehrani F. Androgens stimulate telomerase expression, activity and phosphorylation in ovarian adenocarcinoma cells. Mol Cell Endocrinol 2010;330:10–16.

Novak P, Jensen TJ, Garbe JC, Stampfer MR, Futschek BW. Stepwise DNA methylation changes are linked to escape from defined proliferation barriers and mammary epithelial cell immortalization. Cancer Res 2009;69:5251–5258.

Ogura F, Wako S, Kuroda Y, Tsuchiya K, Bagheri M, Heneidi S, Chazenbalk G, Aiba S, Dezawa M. Human adipose tissue cells possess a unique population of pluripotent stem cells with non-tumorigenic and low telomerase activities: potential implications in regenerative medicine. Stem Cells Dev 2014;23:717–728.

Olenikov AM. [Principle of marginotomy in template synthesis of polynucleotides]. Dokl Akad Nauk SSSR 1971;201:1496–1499.

Olenikov AM. A theory of marginotomy. The incomplete copying of template strands in telomeres of human and human. Science 1971;173:955–959.

Oshita T, Nagai N, Mukai K, Shigemasa K, Hiura M, Ohama K. Telomerase activation in endometriosis and other risk factors. Clin Cancer Res 2014;20:5917–5926.

Park JI, Venteicher AS, Hong JY, Choi J, Jun S, Shkreli M, Chang W, Meng ZJ, Oshita T, Yoon Y. PGC1alpha-mediated repression of the telomerase target gene chromatin. J Biol Chem 2015;290:2259–2269.

Pandita TK, Benvenuto JA, Shay JW. Telomere-associated aging disorders. Ageing Res Rev 2016; doi:10.1016/j.arr.2016.05.009 [In press].

Park JI, Venteicher AS, Hong JY, Choi J, Jun S, Shkreli M, Chang W, Meng ZJ, Oshita T, Yoon Y. PGC1alpha-mediated repression of the telomerase target gene chromatin. J Biol Chem 2015;290:2259–2269.

Petersen S, Sarettzi G, von Zglinicki T. Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. Exp Cell Res 1998;239:152–160.
Pines A. Telomere length and telomerase activity in the context of menopause. *Climacteric* 2013;16:629–631.

Pirvulet V. Gastrointestinal stem cell up-to-date. *J Med Life* 2015;8:245–249.

Plaza-Parracho F, Bacallao K, Poblete C, Gabler F, Carvajal R, Romero C, Valladares L, Vega M. The role of androst-5-ene-3beta,17beta-diol (androstenediol) in cell proliferation in endometrium of women with polycystic ovary syndrome. *Studios S* 2014;49:1–19.

Prianishnikov VA. On the concept of stem cell and a model of functional-morphological structure of the endometrium. *Contraception* 1978;18:213–223.

Radan L, Hughes CS, Teichroeb JH, Vieira Zamora FM, Jewer M, Postovit LM, Betts DH. Microenvironmental regulation of telomerase isoforms in human embryonic stem cells. *Stem Cells Dev* 2014;23:2046–2066.

Rahmanto YS, Jung JG, Wu RC, Kobayashi Y, Heaphy CM, Meeker AK, Wang TL, Shih IM. Inactivating ARID1A tumor suppressor enhances TERT transcription and maintains telomere length in cancer cells. *J Biol Chem* 2016;291:9680–9699.

Rane JK, Greener S, Frame PM, Mann VM, Simmzs MS, Collins AT, Berney DM, Maitland NJ. Telomerase activity and telomere length in human benign prostatic hyperplasia stem-like cells and their progeny implies the existence of distinct basal and luminal cell lineages. *Eur Urol* 2016;69:551–554.

Robinson JD, Ludlow AT, Batten K, Magdiner F, Stadler G, Wagner KR, Shay JW, Wright WE. Telomere position effect: regulation of gene expression with progressive telomere shortening over long distances. *Genes Dev* 2014;28:2464–2476.

Rocchi L, Pacilli A, Sethi R, Penzo M, Schneider RJ, Trere D, Brigotti M, Montanaro L. Dyskerin depletion increases VEGF mRNA internal ribosome entry site-mediated translation. *Nucleic Acids Res* 2013;41:8308–8318.

Roth A, Harley CB, Baerlocher GM. Imetelstat (GRN163L)—telomerase-based cancer therapy. Recent Results Cancer Res 2010;184:221–234.

Royale N. Telomeres: 2nd edn. Cold Spring Harbor Laboratory Press; New York: 2006.

Runge KV, Lustig AJ. Editorial: the evolving telomeres. *Front Genet* 2016;7:50.

Saini N, Srinivasan R, Chowla Y, Sharma S, Chakrabarti A, Rajwanshi A. Telomerase activity, telomere length and human telomerase reverse transcriptase expression in hepatocellular carcinoma is independent of hepatitis virus status. *Liver Int* 2009;29:1162–1170.

Saito T, Schneider A, Martel N, Mazumo H, Bulgy-Moerschel M, Kudo R, Nakazawa H. Proliferation-associated regulation of telomerase activity in human endometrium and its potential implication in early cancer diagnosis. *Biochem Biophys Res Commun* 1997;231:610–614.

Sakamoto M, Toyoizumi T, Kikuchi Y, Okamoto A, Nakayama H, Aoki D, Roth A, Harley CB, Baerlocher GM. Imetelstat (GRN163L). *Aging Cell* 2014;13:376–385.

Shih IM. Inactivating ARID1A tumor suppressor enhances TERT transcription and maintains telomere length in cancer cells. *J Biol Chem* 2016;291:9680–9699.

Shao G, Balajee AS, Hei TK, Zhao Y. p16INK4a downregulation is involved in malignant prostate cancer cells in vitro. *Cancer Discov* 2014;4:161–168.

Seimiya H, Sawada H, Muramatsu Y, Shimizu M, Ohiko K, Yamane K, Tsuruo T. Involvement of 14-3-3 proteins in nuclear localization of telomerase. *EMBO J* 2000;19:2652–2661.

Shimizu Y, Takeuchi T, Mita S, Notsu T, Mizuguchi K, Kyo S. Kruppel-like factor 4 mediates anti-proliferative effects of progesterone with G(0)/G(1) arrest in human endometrial epithelial cells. *J Endocrinol Invest* 2010;33:745–750.

Shroyer KR, Stephens JK, Silverberg SG, Markham N, Shroyer AL, Wilson ML, Enomoto T. Telomerase expression in normal endometrium, endometrial hyperplasia, and endometrial adenocarcinoma. *Int J Gynecol Pathol* 1997;16:225–232.

Shukla S, Schmidt JC, Goldfarb KC, Cech TR, Parker R. Inhibition of telomerase RNA decay rescues telomerase deficiency caused by dyskerin or PARN defects. *Nat Struct Mol Biol* 2016;23:286–292.

Singhapal C, Pal D, Czapiowski R, Porika M, Nelson G, Saretzki GC. Mitochondrial telomerase protects cancer cells from nuclear DNA damage and apoptosis. *PLoS One* 2013;8:e52989.

Smekalova EM, Shibumetskaia OS, Zvereva MI, Gromenko EV, Rubtsova MP, Sourial S, Tempest N, Hapangama DK. Theories on the pathogenesis of endometriosis. *Int J Reprod Med* 2014;2015:179515.

Stadler G, Rahimov F, King OD, Chen JC, Robin JD, Wagner KR, Shay JW, Emerson CP Jr., Wright WE. Telomere position effect regulates DUX4 in human facioscapulohumeral muscular dystrophy. *Nat Struct Mol Biol* 2013;20:671–678.

Takah H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Cancer Discov* 2013;3:1549–1556.

Takah K, Kibe T, Dongjan YR, Frescas D, de Lange T. Telomere protection by TPPI/POT1 requires tethering to TIN2. *Mol Cell* 2011;44:647–659.

Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Cancer Discov* 2013;3:1549–1556.

Takah K, Kibe T, Dongjan YR, Frescas D, de Lange T. Telomere protection by TPPI/POT1 requires tethering to TIN2. *Mol Cell* 2011;44:647–659.

Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Cancer Discov* 2013;3:1549–1556.

Takah K, Kibe T, Dongjan YR, Frescas D, de Lange T. Telomere protection by TPPI/POT1 requires tethering to TIN2. *Mol Cell* 2011;44:647–659.

Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Cancer Discov* 2013;3:1549–1556.

Takah K, Kibe T, Dongjan YR, Frescas D, de Lange T. Telomere protection by TPPI/POT1 requires tethering to TIN2. *Mol Cell* 2011;44:647–659.

Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Cancer Discov* 2013;3:1549–1556.

Takah K, Kibe T, Dongjan YR, Frescas D, de Lange T. Telomere protection by TPPI/POT1 requires tethering to TIN2. *Mol Cell* 2011;44:647–659.

Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Cancer Discov* 2013;3:1549–1556.

Takah K, Kibe T, Dongjan YR, Frescas D, de Lange T. Telomere protection by TPPI/POT1 requires tethering to TIN2. *Mol Cell* 2011;44:647–659.
Ulaner GA, Giudice LC. Developmental regulation of telomerase activity in human fetal tissues during gestation. Mol Hum Reprod 1997;3:769–773.

Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR. Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts. Cancer Res 1998;58:4168–4172.

Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR. Tissue-specific alternate splicing of human telomerase reverse transcriptase (hTERT) influences telomere lengths during human development. Int J Cancer 2001;91:644–649.

Vitaletti S, Vidael J, Guitton A, Robert F, Jaffrès C, Vajda S. Telomerase activity in human endometrial epithelial cells. Protein subunit exchange across the cell cycle. J Biol Chem 2000;275:21320–21325.

Vitaletti S, Vidael J, Guitton A, Robert F, Jaffrès C, Vajda S. Telomerase activity in human endometrial epithelial cells: phenotypic and functional characterization and implications in the pathogenesis of endometriosis. Hum Reprod 2013;28:2695–2708.

Yang C, Przyborski S, Cooke MJ, Zhang X, Stewart R, Anyfantakis G, Atkinson SP, Saretzki G, Armstrong L. A key role for telomerase reverse transcriptase unit in modulating human embryonic stem cell proliferation, cell cycle dynamics, and in vitro differentiation. Stem Cells 2008;26:850–863.

Yeap BB, Knuiman MW, Divitini ML, Hui J, Arscott GM, Handelsman DJ, McLennan SV, Twigg SM, McQuillan B, Hung J et al. Epidemiological and Mendelian randomization studies of dihydrotestosterone and estradiol and leukocyte telomere length in men. J Clin Endocrinol Metab 2016;101:1299–1306.

Zafrakas M, Grimbizis G, Timologou A, Tarlatzis BC. Endometriosis and ovarian cancer in the macaque endometrium. Mol Hum Reprod 2007;13:1107–1110.

Zheng J, Huang F. Stem cell and endometriosis: new knowledge may be producing novel therapies. Int J Clin Exp Med 2014;7:3853–3858.

Zhou C, Bae-Jump VL, Whang YE, Gehrig PA, Boggess JF. The PTEN tumor suppressor inhibits telomerase activity in endometrial cancer cells by decreasing hTERT mRNA levels. Proc Natl Acad Sci U S A 2005;102:12011–12016.

Zhu C, Bae-Jump VL, Whang YE, Gehrig PA, Boggess JF. The PTEN tumor suppressor inhibits telomerase activity in endometrial cancer cells by decreasing hTERT mRNA levels. Proc Natl Acad Sci U S A 2005;102:12011–12016.