Telomerase in the ovary

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Abstract

Telomerase, an enzyme complex that binds the chromosome ends (telomeres) and maintains telomere length and integrity, is present in germ cells, proliferative granulosa cells, germline stem cells, and neoplastic cells in the ovary, but it is absent in differentiated or aged cells. Activation of telomerase in the ovary underpins both benign and malignant cell proliferation in several compartments, including the germ cells, membrana granulosa, and the ovarian surface epithelium. The difference in telomerase operation between normal and abnormal cell proliferations may lie in the mechanisms of telomerase activation in a deregulated manner. Recent studies have implicated telomerase activity in ovarian cancer as well as oogenesis and fertility. Inhibition of telomerase and the shortening of telomeres are seen in occult ovarian insufficiency. Studies of how telomerase operates and regulates ovary development may provide insight into the development of both germ cells for ovarian reproductive function and neoplastic cells in ovarian cancer. The current review summarizes the roles of telomerase in the development of oocytes and proliferation of granulosa cells during folliculogenesis and in the process of tumorigenesis. It also describes the regulation of telomerase by estrogen in the ovary.

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Introduction

Cell proliferation in the ovary is critical to the ovarian functions of producing and releasing germ cells (oocytes) and hormones for reproduction. During the development of oocytes, folliculogenesis is accompanied by significant proliferation of granulosa cells, which is tightly controlled in a temporal and spatial manner. As the overall reproductive lifespan in mammals is predetermined at birth by the number of eggs in each ovary, the ovarian aging manifestations of menopause occur when the finite supply of eggs is depleted. The timing when ovarian aging begins is critical and often reflected by the speed of primordial follicle depletion. The magnitude of the follicular reserve is determined by a discontinuous balance between follicular recruitment, sustainment, and exhaustion (McGee & Hsueh 2000, Adhikari & Liu 2009). In follicular recruitment and sustainment, ovarian cell proliferation plays a primary role, with granulosa cell proliferation, differentiation, and subsequent senescence (aging) and apoptosis (death) to underpin oogenesis and the production of estrogen and progesterone. Recent studies have shown the presence of the cells in adult ovaries with germline stem cell properties of proliferative potential in the postnatal mammalian ovary (Virant-Klun *et al.* 2009, Zou *et al.* 2009). Implantation of a cell line established from the germline stem cells of adult mouse ovary into the ovaries of infertile mice reinitiates oogenesis and

fertility (Zou *et al.* 2009). The presence of germline stem cells in adult ovary may reflect suggesting the presence of a potentially renewal mechanism in support of the ovary reproduction, and release of eggs and hormones.

Several lines of evidence indicate that specific genetic mechanisms mediate production by the ovary of germ cells and the hormones needed to support oogenesis. The ovary is surrounded by a single layer of ovarian surface epithelium (OSE) that is a layer of cuboidal cells derived from mesoderm during embryonic development (Auersperg *et al.* 2001). As a source of ovarian stem cells in humans (Virant-Klun *et al.* 2009) and mice (Lee *et al.* 2008), OSE undergoes hyperplasia in response to estrogen treatment to potentially lead to tumorigenesis (Laviolette *et al.* 2010). Estrogen accelerates tumorigenesis initiated by the simian virus 40 (SV40) T antigens, resulting in an early onset and reduced overall survival time (Laviolette *et al.* 2010). In the follicular compartment, estrogen is released by and acts on folliculogenesis. In contrast, recent studies showed that phosphatase and tensin homolog (PTEN) that binds and dephosphorylates the phosphatidylinositol (3,4,5)-trisphosphate has a chief function in suppressing folliculogenesis and ovulation (Fan *et al.* 2008, Marx 2008, Reddy *et al.* 2008, Adhikari & Liu 2009). Lacking PTEN in oocytes causes the entire primordial follicle pool to become activated, followed by depletion of all primordial follicles in young adult mice (Reddy *et al.* 2008, Gleicher *et al.* 2009),

whereas inactivating PTEN in the granulosa cells results in not only enhanced ovulation but also persistent non-steroidogenic luteal structures in the adult mouse ovary (Fan *et al.* 2008). To date, however, neither the downstream signaling or effector of PTEN, nor the opposing positive regulatory pathway in follicular activation is known.

One of the major genetic mechanisms determining cell proliferative capacity is the maintenance of telomeres. Work from several independent laboratories has shown that forced expression of the telomerase reverse transcriptase (*TERT*) gene to maintain telomeres in cultured OSE cells results in increased proliferative potential of the cells, causing them to resemble stem cells (Davies *et al.* 2003, Kusakari *et al.* 2003, Alvero *et al.* 2004, Maeda *et al.* 2005, Li *et al.* 2007, Yang *et al.* 2007a, 2007b). This fundamental effect of telomere remodeling on OSE cells demonstrates proof of principle that the regulation of telomere remodeling is closely linked to OSE cell proliferative capacity. However, substantial *in vivo* evidence also shows that telomere remodeling is regulated by various factors, including hormones, growth factors, and cytokines, under diverse conditions. Intriguingly, during folliculogenesis, ovarian aging, and neoplastic development, the pattern of TERT-induced telomerase activity is dependent on cell type, time, and location. This review presents the current understanding of the fundamental roles of telomerase and telomere maintenance in ovarian cell proliferation.

Telomere and telomerase: a mechanism for cell regeneration

Telomeres, the specialized structures at the ends of all eukaryotic chromosomes, are formed from a unique DNA nucleotide sequence and specific coat proteins. Telomeres from different species assume various tertiary and quaternary structures. The telomeric DNA sequence in humans comprises extended arrays of a few thousand tandem repeats of TTAGGG complementary double strands. However, telomeres end with a single-stranded 3'-end overhang of ~150 bp. This 3' single-stranded overhang is protected from being sensed as DNA breaks by protein complexes or within the double-stranded duplex of the telomere (de Lange 2005, Zhao *et al.* 2008, Lue 2009; Fig. 1). Entry of the telomere 3' overhang into the duplex results in a displacement loop and a larger telomere loop at each end of the chromosome (Griffith *et al.* 1999). The specialized configuration of telomeric DNA has a critical role in protecting chromosomes. During each round of telomeric DNA replication, telomeres are shortened due to the end replication problem (Olovnikov 1996). With each round of chromosome replication, 100–200 bp of telomeric DNA are lost; so, telomeres shorten from 10–15 to 2–5 kb in a lifespan of human cells. When telomeres shorten

Figure 1 Simplified cartoon structures of linear and circular telomeres at the ends of chromosomes. Double-stranded telomere DNAs are bound by telomeric repeat-binding factor 1 (TRF1, also known as TERF1) and TRF2 (TERF2). The 3' single-stranded overhang of telomere is capped by telomerase complex (circled; which is composed by TERT and TERC). The long telomere hides its 3' single-stranded overhang in the telomere double-stranded DNA duplex, resulting in a local displacement loop (D-loop) as indicated and a large telomere loop (T-loop). Symbol keys are indicated for TRF1, TRF2, TERT, and TERC.

to a critical length, the cell exits the cell cycle, which is characteristic of cell senescence (Counter *et al.* 1992, Shay 1999, Blackburn 2001, Wong & Collins 2003). Thus, the initial size of telomeres and the extent of telomere shortening during cell divisions define a cell's replicative lifespan.

The telomere end replication problem is evolutionarily solved by the enzyme complex telomerase (Szostak & Blackburn 1982, Greider & Blackburn 1985). Telomerase binds, protects, and positively regulates telomere length (Fig. 1). Telomerase is conserved from yeast to higher eukaryotes to synthesize telomeres by RT and to maintain telomeres for continuous renewal and proliferation, particularly of highly proliferative cells, such as germ lines and stem cells in mammals (Shay 1999, Blackburn 2001). As a ribonucleoprotein complex, telomerase contains the catalytic subunit TERT, the telomerase RNA components (TERC), and binding proteins (Cohen *et al.* 2007, Venteicher *et al.* 2009). Murine TERT comprises 1122 amino acid residues encoded by a gene on chromosome 13 (D13Mit8; Greenberg *et al.* 1998). Murine TERC comprises 430 nucleotides (Blasco *et al.* 1995). Reconstitution of telomerase demonstrates that TERT is the rate-limiting factor capable of lengthening telomeric DNA when expressed in cells (Bodnar *et al.* 1998). Expression of TERT enables telomerase to function in telomere lengthening and length maintenance; conversely, deficiency of either TERT or TERC abolishes telomerase activity and results in short telomeres (Fig. 2).

Telomerase is required for oocyte development

Telomerase is present in oocytes from early antral and preovulatory follicles, as well as in ovulated oocytes, and at markedly decreased levels during oocyte maturation

Figure 2 Model of the mechanisms by which estrogen stimulates telomerase activity, telomere lengthening, and tumorigenesis in the ovary. (A) Estrogen (E₂) is produced by granulosa cells (GC) under the action of GNRH and FSH, and the autocrine actions of estrogen. Estrogen stimulation of telomerase (TE) activity underlies GC proliferation. (B) Oversupply of estrogen induces high telomerase activity, lengthening of telomeres, and GC tumor formation. (C) Spillover of estrogen to the epithelial compartment causes telomerase activation, telomere lengthening, and ovarian carcinoma.

(Eisenhauer *et al.* 1997, Betts & King 1999). Knock out in mice of TERT (Leri *et al.* 2003, Wong *et al.* 2003) or TERC (Blasco *et al.* 1997, Hande *et al.* 1999) eliminates telomerase activity, compromises telomere maintenance, and results in defects of cell developments in multiple tissues. In later generations of TERC-deficient mice, highly proliferative tissues such as those of the reproductive organs, immune system, hematopoietic system, and the germ line show compromised proliferative capacity (Blasco *et al.* 1995, 1997, Lee *et al.* 1998). Infertility is a major phenotype of late generations of telomerase-deficient mice (Lee *et al.* 1998, Herrera *et al.* 1999). A significant decline in litter size occurred by the fourth generation, when telomeres are markedly shortened, and no progeny was produced from eight different intercrosses of sixth-generation mice (Lee *et al.* 1998, Herrera *et al.* 1999). The uterine horns of infertile mice lacking TERC are significantly shorter in length and diameter, and the ovarian tissues are considerably smaller (Lee *et al.* 1998). Histological analysis confirmed the reduced size of the TERC-deficient ovary, and also revealed reduced myometrial thickness with increased atrophy of the smooth muscle cells in the uterine horns (Lee *et al.* 1998). Intercrosses between wild-type male and TERC-deficient female mice produced an average of 3.9 ± 2.9 fertilized eggs per TERC-deficient female compared to 9.8 ± 3.8 per female in wild-type litters (Lee *et al.* 1998). The atrophic uterus and compromised ovulation are indicative of decreased steroid hormone production by granulosa cells and decreased oocyte development in telomerase-deficient mice.

High levels of telomerase activity are present in germline stem cells of ovarian origin (Virant-Klun *et al.* 2009, Zou *et al.* 2009), which have the capacity to differentiate into oocytes on implantation into the mouse ovary and confer fertility on infertile ovaries (Zou *et al.* 2009). Consistently, human ovarian germline stem cells with high telomerase activity (scraped from 21 post-menopausal women) proliferate *in vitro* to produce

oocyte-, blastocyst-, and embryo-like structures (Virant-Klun *et al.* 2009). The human embryonic stem cell lines H9 and hES-NCL1 have been used for differentiation to primordial germ cells (Tilgner *et al.* 2008). Similarly, mouse embryonic stem cells have been shown to form ovarian follicles and oocyte-like cells (Qing *et al.* 2007, Virant-Klun *et al.* 2009). A stem cell-like line with high telomerase activity has been established from preantral follicle cultures from 35 mouse blastocysts from 193 parthenotes (Lee *et al.* 2008). Mouse embryonic bodies cultured in testis-conditioned medium developed into ovarian structures containing putative oocytes that expressed oocyte-specific markers such as FIGLA (Fig- α) and ZP3, and were surrounded by one to two layers of flattened cells without a visible zona pellucida (Lacham-Kaplan *et al.* 2006). Collectively, these studies demonstrate that telomerase activity is important in the processes of oocyte development and parthenogenesis.

In determining the effects of telomerase deficiency on germ cell development, researchers have investigated the cleavage and pre-implantation development of embryos derived from *in vivo* and IVF of sperm with oocytes with or without TERC (Liu *et al.* 2002). Fertilization of oocytes containing short telomeres by TERC wild-type sperm leads to aberrant cleavage and development of the embryos. More than 50% of the fertilized eggs developed only one pronucleus, and had a high incidence of cytofragmentation (Liu *et al.* 2002). These data suggest that short telomeres in the oocytes contribute to anomalous fertilization of gametes and abnormal cleavage of embryos. The defects of short telomeres from oocytes appear not to be reset sufficiently following fertilization. So despite significant telomere lengthening during early cleavage development, the data showing that telomere repair is insufficient in the absence of telomerase argue in favor of a significant role for telomerase in the reprogramming of telomere length after fertilization. Under normal conditions, oocytes have shorter telomeres than somatic cells; so

telomere reprogramming for lengthening must take place following fertilization. Telomeres are reprogrammed by a recombination-based mechanism during early cleavage cycles, and from the blastocyst stage onwards, telomerase maintains the telomere length established by this alternative mechanism (Liu *et al.* 2007). Telomerase activity is present at low levels in ovulated oocytes, significantly increased in four-cell rat embryos following fertilization, bovine zygotes, and two- to five-cell embryos, but decreased in six- to eight-cell embryos, morulae, and blastocysts (Eisenhauer *et al.* 1997, Betts & King 1999, Xu & Yang 2000). Thus, following fertilization, the dramatic telomere remodeling involves both telomerase activity and telomere homologous recombination to enable embryonic development.

Role of telomerase in granulosa cell proliferation during folliculogenesis

Granulosa cells share many characteristics with other epithelia, including their origin as epithelial stem cells. In developing ovarian follicles, where intense cell division is crucial to sustain the estrous cycle and drive oocyte development, the formation and regression of granulosa cells determines the development of germ cells and also the timing and the amount of hormones secretion. Studies have consistently shown that high telomerase activity is present in the smallest preantral follicles, and telomerase activity declines gradually during the transition from small- to medium-sized follicles (Lavranos *et al.* 1999). *In situ* hybridization and *in situ* assay of telomerase activity demonstrate the association of high levels of telomerase activity with the highly proliferative granulosa cells in growing follicles (Lavranos *et al.* 1999, Yamagata *et al.* 2002), suggesting that the high proliferative activity of granulosa cells depends on telomerase activity to maintain telomeres. Following ovulation, the follicle develops into the corpus luteum with the granulosa cells to exit the cell cycle undergoing differentiation to luteal cells and apoptosis. Recently, luteinizing granulosa cells isolated from the ovarian follicles of infertile patients have been shown to survive in cultures for a long period of time, and a subset of the differentiated granulosa cells shows *in vitro* differentiation into other cell types such as neurons, chondrocytes, and osteoblasts (Kossowska-Tomaszczuk *et al.* 2009). In the large follicles undergoing atretic changes, however, telomerase activity is significantly less, and the inhibition of telomerase is prevented by estrogen administration in rats, suggesting that telomerase withdrawal plays an integral role in granulosa cell apoptosis and follicular atresia induced by estrogen withdrawal (Yamagata *et al.* 2002).

Analysis (by immunohistochemistry and fluorescence *in situ* hybridization) of the distribution of TERT during folliculogenesis and its correlation with telomeres in

porcine ovarian primary and preantral follicles showed that TERT is expressed in granulosa cells with a typical nuclear location, and that its expression correlates with the size of telomeres. Long telomeres were seen from preantral to antral follicles (Russo *et al.* 2006). Telomerase activity was detected in two distinct populations of porcine granulosa cells: relatively undifferentiated granulosa cells isolated from small (1–2 mm) antral follicles and differentiated cells with advanced functionality obtained from large (5–7 mm) antral follicles (Tomanek *et al.* 2008). However, epidermal growth factor stimulated telomerase activity to a greater extent in the granulosa cells isolated from small follicles (Tomanek *et al.* 2008), suggesting that telomerase activity and telomere lengthening are needed for granulosa cell proliferation, particularly in small, rapidly growing follicles. A cross-sectional study on 54 women ≤ 37 years of age undergoing IVF with classified occult ovarian insufficiency investigated telomerase activity and telomere length in the granulosa cells acquired at the time of oocyte retrieval (Butts *et al.* 2009). Lack of granulosa cell telomerase activity and short telomeres were found, suggesting that aberrant telomere homeostasis is associated with occult ovarian insufficiency in young women (Butts *et al.* 2009).

Thus, abnormalities in telomere length and telomerase activity in human granulosa cells may serve as molecular markers for occult ovarian insufficiency (Butts *et al.* 2009). Compromised telomerase maintenance of telomeres in granulosa cells might play a causal role in infertility due to occult ovarian insufficiency. The age-related decline in ovarian follicle number has been attributed to estrogen deficiency, but the cause of failed maintenance of telomeres in occult ovarian insufficiency in young women is poorly understood.

Regulation of telomerase activity by estrogen

The ovary is both a major source of estrogen and an important target organ of the hormone. By autocrine mechanisms, estrogen exerts profound effects on the ovary (Weihua *et al.* 2003). Withdrawal of estrogen induces structural atrophy and dysfunction of the ovaries. Conversely, prolonged exposure to estrogen leads to the risk of certain types of tumors, such as ovarian cancer (Lacey *et al.* 2002). Estrogen acts by binding to estrogen receptors (ESR) that dimerize and bind to estrogen response elements (ERE) in the promoters of estrogen target genes to regulate gene transcription (Bjornstrom & Sjoberg 2005). The estrogen–ESR complex also transactivates gene expression through interactions with other DNA-binding elements (Huang *et al.* 2004). Estrogen acts mainly through ESR1 (ER α) and ESR2 (ER β ; Couse *et al.* 2000), two members of the nuclear receptor superfamily. Both ESR forms are present in granulosa cells of the rodent ovary, with ESR2 suggested to be the predominant form (Palter *et al.* 2001). While ESR2 is essential for granulosa

cell proliferation, ESR1 has specific roles in ovulation, corpus luteum formation, and interstitial glandular cell development (Couse *et al.* 2000, Dupont *et al.* 2000). In addition, both ESR1 and ESR2 are implicated in regulating *TERT* gene transcription in cancer and healthy differentiated cells (Kondoh *et al.* 2007, Grasselli *et al.* 2008).

Estrogen activates *TERT* transcription by a direct action on the gene in ESR-positive human cancer cell lines (Kyo *et al.* 1999, Misiti *et al.* 2000, Ling *et al.* 2006). Moreover, in human OSE cells, a putative ERE identified in the *TERT* gene promoter binds ESR1 (Misiti *et al.* 2000). *In vivo* DNA footprinting showed an ESR-dependent remodeling of the *TERT* gene promoter in OSE cells (Misiti *et al.* 2000). Estrogen and ESR1, but not ESR2, markedly stimulate *TERT* gene promoter activity in an ERE-dependent manner; the estrogen-induced telomerase activity occurs within 3 h of treatment of OSE cells (Misiti *et al.* 2000).

Although both ESR1 and ESR2 are present in murine ovary, the mouse *TERT* promoter does not appear to possess a canonical ERE. Estrogen stimulates the expression of transcription factor MYC (c-myc) in rat granulosa cells *in vivo* (Piontekewitz *et al.* 1997). The *MYC* gene is likely to be a direct target of ESR, as stimulation of *MYC* gene transcription occurs within 1 h of estrogen exposure (Santos *et al.* 1988). Estrogen stimulation of telomerase activity is dependent on the integrity of the *MYC* gene in human choriocarcinoma cells (Sarkar *et al.* 2006). These data suggest that estrogen positively regulates *TERT* gene transcription by both a direct action on the *TERT* gene promoter and an indirect mechanism involving the *MYC* gene. In ovarian cancers, *MYC* mediates aurora-A kinase-induced telomerase activity (Yang *et al.* 2004), and the actions of *MYC* on the *TERT* gene are further controlled by the tumor suppressor BACR1 (Zhou & Liu 2003) and the transforming growth factor- β family signaling transducer SMAD3 (Li & Liu 2007, Cassar *et al.* 2009, Liu *et al.* 2010).

Evidence suggests that prolonged exposure of the ovary to high levels of estrogen is associated with the development of ovarian tumors. Estrogen replacement therapy may also be involved in promoting ovarian cancer (Lacey *et al.* 2002). The mitogenic effects of estrogen have been studied in various ESR-positive ovarian cancer cell lines, and anti-estrogens, such as tamoxifen and glyceollins, suppress ovarian cancer cell growth *in vitro* and in animal models. Moreover, estrogen induces ovarian papillary neoplasms in several species, including dogs (Jabara 1962), rabbits (Bai *et al.* 2000), and pigs (Silva *et al.* 1998). Expression of ESR even at low levels has been associated with an earlier-stage, higher tumor differentiation and better prognosis (Geisler *et al.* 1996, Munstedt *et al.* 2000). These *in vitro* and *in vivo* studies support the notion that estrogen plays a significant part in ovarian tumorigenesis by regulating the genes underlying tumor cell immortalization and transformation. Further studies are required to investigate

the mechanism by which estrogen induces tumorigenesis. During multi-step ovarian carcinogenesis, many alterations occur in the interactive network of estrogen-responsive genes, including the proto-oncogene *MYC*, anti-apoptotic protein *BCL2*, and *TERT* (Chow *et al.* 1996). These estrogen-regulated genes may play important roles during ovarian tumorigenesis, such as aberrant epithelial cell proliferation, immortalization, transformation, invasion, and metastasis (Li *et al.* 2008; Fig. 1).

Reactivation of telomerase in OSE and the role in ovarian regeneration and oncogenesis

The replicative lifespan is limited in cells lacking telomerase activity by replication-associated telomere shortening. Therefore, in the context of ovarian regeneration, telomerase activation might extend the lifespan of replicating OSE and other ovarian progenitor cells. On the other hand, high levels of telomerase activity are a hallmark of cancer, including ovarian epithelial carcinoma. Accumulating data indicate that telomerase activation is an early event in ovarian carcinogenesis. At present, it is unclear whether telomerase activation preserves the non-malignant phenotype and replicative longevity of ovarian reproductive cells or constitutes an early alteration needed for subsequent unlimited proliferation and malignant transformation.

Increasing telomerase activity by overexpressing *TERT* alone (Alvero *et al.* 2004), *TERT* plus SV40 large T antigen (Davies *et al.* 2003, Kusakari *et al.* 2003), or *TERT* plus human papillomavirus-16 antigens (Maeda *et al.* 2005) extended the proliferative lifespan of human OSE cells toward immortality. These studies independently showed the requirement for inactivation of the p53 and RB1 (Rb) tumor suppressor mechanisms in *TERT*-induced cell immortalization. To establish whether a single disruption of either the p53 or RB1 pathway would promote telomerase-induced human OSE cell immortalization, Liu *et al.* used RNAi technology to knock down either RB1 or p53 in combination with ectopic expression of the *TERT* in human OSE cells. Significantly, knockdown of either RB1 (Yang *et al.* 2007a) or p53 (Yang *et al.* 2007b) in the presence of *TERT* is sufficient to immortalize human OSE cells. It was later shown that human OSE cells could be immortalized by *TERT* expression without functional disruption of RB1 and p53 (Li *et al.* 2007). Thus, while telomerase activity is indispensable in the immortalization step of human OSE, p53 and RB1 are not, at least under certain conditions. Furthermore, *TERT*-immortalized human OSE cells have lengthened telomeres, are diploid without chromosomal instability, and maintain epithelial characters without tumorigenicity (Kusakari *et al.* 2003, Yang *et al.* 2007a, 2007b, Sasaki *et al.* 2009).

The importance of telomerase in tumorigenesis has been fully recognized (Shay & Bacchetti 1997,

Shay & Wright 2005). Telomerase activity and dysfunctional telomeres have been demonstrated in human ovarian carcinoma (Counter *et al.* 1994, Baykal *et al.* 2004). Telomerase is not present in normal OSE and premalignant lesions, but is up-regulated in 90–97% of ovarian carcinomas (Gorham *et al.* 1997, Yokoyama *et al.* 1998, Baykal *et al.* 2004, Bayne & Liu 2005, Bayne *et al.* 2007, Shay & Wright 2007). By *in situ* hybridization, 90% (28 of 31) of ovarian carcinomas expressed *TERT*. However, *TERT* gene expression has also been found in benign epithelial tissues, so it is unlikely to be a useful marker for differentiating between benign and malignant tumors (Baykal *et al.* 2004).

Ovarian cancer is highly lethal. Based on the pattern of epithelial differentiation and morphological criteria, there are four major subtypes of ovarian epithelial cancer: serous, mucinous, endometrioid, and clear cell. Serous tumors are most common, representing about 50% of all ovarian cancers derived from epithelial cells. Endometrioid adenocarcinomas account for 20–25% of ovarian cancer, and clear cell and mucinous adenocarcinomas account for <10% of ovarian cancer (Soslow 2008). Aggressive ovarian tumors result from the uncontrolled proliferation of ovarian stem cells (Bapat *et al.* 2005, Szotek *et al.* 2006), and epithelial ovarian cancer is thought to arise from OSE (Young *et al.* 2004). Consistently, *TERT*- and SV40 large T antigen-immortalized human OSE cells undergo anchorage-independent growth *in vitro* and tumorigenesis in mice upon forced expression of either oncogene *ERBB2* (*c-erbB-2*) or mutant *HRAS* (Ha-Ras; Kusakari *et al.* 2003). Recently, oncogenic transformation of human OSE cells has been investigated with defined cellular oncogenes (Sasaki *et al.* 2009). Non-viral transduction of primary human OSE cells with human genes encoding mutant cyclin-dependent kinase 4, cyclinD1, and *TERT* establishes normal diploid OSE cells with extended proliferation without chromosomal instability (Sasaki *et al.* 2009). Inactivation of p53 plus oncogenic transduction of the *KRAS* oncogene did not lead to tumor formation, but the additional transduction of *AKT*, or combined transduction of *MYC* with *BCL2*, resulted in tumor formation (Sasaki *et al.* 2009). The findings of Sasaki *et al.* (2009) demonstrate that the presence of telomerase, together with tumor suppressor p53, proto-oncogenes *RAS* and *MYC*, and the anti-apoptotic pro-survival *BCL2*, causes the development of ovarian epithelial cancer *in vitro*.

The future

The discoveries from various studies indicating that telomerase has an indispensable role in ovarian germ cell development highlight a regulatory mechanism through which telomerase determines different cell proliferative potentials by regulating ovarian telomere homeostasis. The regulation of telomerase activity may thus provide a means to modulate germ cell

development in the ovary. The regulation of telomerase activity by estrogen implies a pathway through which telomere length and cell proliferative potential can be modulated to support the various functions of the ovary. The regulation of the telomerase–telomere axis by estrogen may also reflect how granulosa cells facilitate germ cell development and how aging occurs when estrogen is withdrawn. Further studies are required to determine a potential intermediate role of telomerase activity in estrogen deficiency-induced ovarian aging. Elucidation of a temporal and spatial mechanism of estrogen regulation of telomerase activity (and the roles of other hormones in telomere remodeling) would provide a basis for the design of interventions to treat ovarian aging and infertility.

However, it is clear that telomerase is a double-edged sword in germ cell development and neoplastic immortalization. The ability to control telomerase activity in OSE could allow the prevention and treatment of ovarian cancers derived from these cells. Multiple signaling molecules and pathways could be involved in telomerase activation during ovarian cancer cell immortalization. They warrant investigation of their relationships to OSE cell immortalization and transformation as seen in ovarian cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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