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Telomeres and aging-related meiotic dysfunction in women

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Abstract. Meiotic dysfunction increasingly afflicts women as they age, resulting in infertility, miscarriage and handicapped offspring. How aging disrupts meiotic function in women remains unclear, but as women increasingly delay childbearing, this issue becomes urgent. Telomeres, which mediate aging in mitotic cells, may also mediate aging during meiosis. Telomeres shorten during DNA replication. In mammals, oocytes remain quiescent, but their precursors replicated during fetal oogenesis. Moreover, eggs ovulated from older women entered meiosis later during fetal oogenesis than eggs ovulated when younger, and therefore underwent more replications. Telomeres also shorten from reactive oxygen, which triggers a DNA repair response, so the prolonged interval between fetal oogenesis and ovulation in some women would further shorten telomeres. Mice normally do not exhibit age-related meiotic dysfunction (interestingly, their telomeres are manyfold longer than telomeres in women), but genetic or pharmacologic shortening of mouse telomeres recapitulates the reproductive aging phenotype of women. This has led to a telomere theory of age-related meiotic dysfunction in women, and underlined the importance to human health of a mechanistic understanding of telomeres and meiosis.

Keywords. Telomeres, meiosis, aging, reproduction, aneuploidy, infertility.

Introduction: aging-related meiotic dysfunction in women

Women experience a marked increase in meiotic dysfunction with age, beginning at least 15 years before menopause, while they still have regular menstrual cycles. Fertility begins to decline in women by their mid-30s, and conceptions result in high rates of miscarriage and/or aneuploid offspring [1–4]. Indeed, meiotic non-disjunction in the oocyte is the single most common genetic cause of mental retardation in children [1]. The clinical and public health importance of aging-related meiotic dysfunction increases as more women delay attempts at child-bearing to pursue careers. Moreover, the emotional, medical and social costs of aneuploid offspring represent a major burden to society, so elucidation of the mechanism of aging-related meiotic dysfunction is an urgent priority.

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Biological basis of aging-related non-disjunction in women

Most aneuploid oocytes and embryos typically undergo apoptosis before implantation, producing infertility [1–5], but some escape only to miscarry later during development or give rise to a handicapped offspring. A number of abnormalities are thought to predispose oocytes from older women to meiotic non-disjunction, including reduced chiasmata and recombination [1, 6], structural abnormalities of the meiotic spindle-chromosome complex [7], mitochondrial DNA (mtDNA) mutations [8–10], and late exit from a production line during fetal oogenesis [11–13]. How aging affects such diverse cellular processes in an orderly fashion in virtually every woman, even while the rest of her organs continue to function at near peak performance, remains an enigma. Interestingly, experimental telomere shortening in the mouse, which normally has long telomeres and exhibits none of the above abnormalities, recapitulates virtually the entire reproductive aging phenotype (see below). Genetic or pharmacologic shortening of telomeres produces

oocytes with reduced chiasmata and synapsis [14], abnormal spindles with abnormal chromosome alignment [15] and arrested embryonic development ending in apoptosis [16, 17]. Moreover, reactive oxygen, such as would be expected to result from even low levels of mtDNA mutations, further shortens telomeres [18]. We have proposed that telomere dysfunction in eggs from older women provides a unifying and parsimonious theory to explain how aging predisposes oocytes from older women to non-disjunction [19].

The biology of telomeres

Telomeres are repeated sequences of TTAGGG, and associated proteins, with a 3′ overhang, which forms a loop to cap chromosome ends and prevents end-to-end fusions. In most cells, telomeres shorten with each cell division and from exposure to reactive oxygen. When critically short, they trigger cell cycle arrest, apoptosis and promote genomic instability [20–22]. The tendency of telomeres to shorten with each cell division and from exposure to reactive oxygen [23–25] makes them a promising candidate to explain the effects of aging on many long-lived cells.

Telomeres have an especially important role in meiosis, because during early meiosis they tether chromosomes and facilitate the alignment, pairing, synapsis and formation of chiasmata [26–29]. During early meiosis telomere 'bouquets' form with a stalklike attachment to the nuclear envelope to facilitate the homologue alignment required for formation of chiasmata. Elucidation of the molecular basis of telomere movement, and specifically the regulation of bouquet formation and resolution, is an active area of investigation, and is discussed elsewhere in this multi-author review.

Telomerase, a reverse transcriptase, can prevent telomere shortening in some cells, especially male germ cells, stem cells and cancer cells. But telomerase activity remains low in female germ cells from the oocyte to the blastocyst stages of development in mammals [30]. Thus, in mammals, female gametes are endowed with their telomere length during early development. Interestingly, in the male, whose reproductive function does not decline appreciably with age, spermatogonia persist as a kind of stem cell in the testes, which expresses telomerase activity throughout the life of the animal. Since telomeres play important roles in chiasmata formation, spindle structure, cell division and cell death, and become increasingly dysfunctional with age, they could play an important role in the age-related meiotic dysfunction seen in women.

Mechanisms of telomere shortening

Telomeres shorten by at least two mechanisms, replicative senescence [21, 22] and via a damage response to reactive oxygen [23]. Once the telomere reaches a critically short length, the cell stops dividing and eventually undergoes apoptosis and/or chromosomal fusions. A few cell types, including stem cells and cancer cells, can avoid replicative senescence by expressing telomerase, a reverse transcriptase, which adds telomeric sequence [20–22].

Non-dividing cells also can loose telomeres when oxidation prompts a DNA damage response, which excises the oxidized sequence [23]. Telomeres are especially susceptible to reactive oxygen-induced, replication-independent telomere shortening because of their guanine-rich sequence (the nucleotide most susceptible to oxidation), location in the nuclear membrane, which facilitates lipid peroxidation, and their relative lack of protective proteins.

Both replicative senescence and reactive oxygen species (ROS)-induced shortening occur in mouse oocytes and early preimplantation embryos [18], the developmental stages when most non-disjunction occurs in older women [1–3]. Replicative senescence at first seems an unlikely process in a cell, such as the oocyte, which does not divide. However, female germ cells do divide, during fetal life, before entry to meiosis. Indeed, DNA labeling studies have shown that the first oocytes formed during fetal oogenesis are the first to ovulate in the pubertal female, and the last oocytes formed are the last to ovulate [10–12]. Telomerase is inactive in oocytes and late preimplantation-stage embryos [30], and telomeres likely further shorten from exposure to reactive oxygen, produced by cellular metabolism during the prolonged interval between the birth of oocytes and their ovulation – up to 45 years in some women. Thus they provide a cytogenetic substrate to explain the 'two hits' of aging on the female reproductive system, one active during fetal life and the other during adult life.

Telomeres, chiasmata and synapsis during meiosis in mammals

In mammals, oocytes initiate meiosis during fetal development, which consists of pairing, synapsis and recombination, followed by protracted meiotic arrest. Chiasmata formed during fetal life influence chromosome disjunction during the preovulatory phase of the adult [1]. During the first meiotic division (MI), the chiasmata formed during fetal oogenesis tether homologous chromosomes. Their deficiency predisposes to subsequent non-disjunction and aneuploidy [1, 30–32], presumably because chiasmata are needed to counter the poleward pulling forces exerted by the meiotic spindle until all chromosomes

have congressed to the metaphase plate. Indeed, children with Down's syndrome, arising from non-disjunction of chromosome 21, have decreased genetic recombination compared with normal children [31, 32].

Mice rarely exhibit age-related meiotic dysfunction, and interestingly, their telomeres are almost an order of magnitude longer than telomeres in humans. However, mice with shortened telomeres, produced by induction of null mutation to the RNA template of telomerase, have markedly reduced chiasmata and synapsis than animals with normal telomere length [14]. Paucity of chiasmata, coupled with chiasmata-independent effects of short telomeres, predispose embryos to genomic instability, cell cycle arrest and apoptosis, the hallmarks of reproductive aging in women.

Telomeres, metaphase chromosome alignment and meiotic spindles

Eggs from older women exhibit abnormalities in chromosome alignment and spindle morphology [6]. Structural abnormalities in the meiotic spindle, including asymmetry of the spindle poles, and failure of chromosomes to align on the metaphase plate appear in almost 80% of eggs aspirated from women over age 40 compared with only 17% of eggs from younger controls.

Shortening of telomeres in mice, which occurs after several generations following telomerase deficiency [15], or by exposure to pharmacologically induced reactive oxygen [16], produces abnormal meiotic spindles with the same asymmetry and congression failure common to eggs from older women [6]. These spindle-chromosome abnormalities would be expected to be especially disruptive to meiosis, since checkpoints for meiotic chromosome at metaphase-to-anaphase transition are less efficient in females than males [1, 33]. During meiosis in females, progression from the MI to MII stages occurs even in the presence of chromosome misalignment at MI, because female meiosis lacks efficient metaphase checkpoint control [33].

The mechanisms underlying chromosome misalignment and disruption of meiotic spindles caused by telomere dysfunction are not well understood, but could result from improper homologous chromosome pairing and recombination during early meiosis. That female Mlh 1 mutant mice, which lack normal recombination, also exhibit abnormal spindle assembly [34] is consistent with this hypothesis. Abnormal chromosome pairing from telomere loss would disrupt pairing and recombination of homologous chromosomes during the leptotene/zygotene stages of prophase I, prohibit organization and maintenance of functional meiotic spindles, and prevent normal chromosome alignment at metaphase during meiotic division.

Mitochondrial dysfunction shortens telomeres

Mitochondrial dysfunction and oxidative stress have been implicated in cellular senescence, apoptosis, aging and aging-associated pathologies, including reproductive senescence [35, 36]. Uncoupling mitochondrial electron transport with pharmacologic agents administered to one-cell embryos disrupts mitochondrial membrane potential ($\Delta\psi m$) and increases ROS production, measured by CM-H2DCFDA. It also compromises embryo development in a time- and dose-dependent manner [18].

Measurement of telomere length in individual oocytes and embryos, by quantitative fluorescence in situ hybridization (Q-FISH) [37, 38], demonstrates that mitochondrial dysfunction causes telomere attrition and eventually chromosome fusions and apoptosis. Therefore, reactive oxygen production can contribute to telomere attrition and genomic instability. The antioxidant, N-acetylcysteine (NAC), forms cysteine by deacetylation, quenches reactive oxygen and increases glutathione levels, which itself scavenges free radicals. NAC prevents the accumulation of reactive oxygen, telomere shortening and cell death induced by the mitochondrial uncouplers, FCCP [18] or arsenite [39]. However, NAC does not rescue embryos from telomere attrition, cell cycle arrest and cell death if administered only after FCCP treatment and extensive oxidative damage.

Mutations in the D-loop of mtDNA, and 5-kb deletions of a region flanked by 13-bp direct repeats, the so-called common deletion, have been found in oocytes from older women [7–9]. The impact of these mutations on energy metabolism in oocytes and early embryos must be minimal, however, since mutant mtDNA represents only a tiny fraction of the more than 100,000 mitochondria transmitted through the cytoplasm of each egg [40]. While energetic dysfunction would be unlikely with such a low burden of mutant mtDNA, they could exert dominant negative effects on development by uncoupling oxidative phosphorylation (oxphos) and producing reactive oxygen, which preferentially attacks the more vulnerable, guanine-rich telomeres.

Telomere length of spare human eggs predicts pregnancy following IVF

Experimentally induced telomere dysfunction can disrupt meiotic function in mice, which normally are nearly completely immune from the effects of telomere erosion. But what about telomere dysfunction in human eggs? Can measurement of telomere length identify eggs from women predisposed to meiotic dysfunction? This is an important question, since age itself provides only a crude predictor of aneuploidy risk. Significant variation exists in the risks of producing aneuploid offspring, even

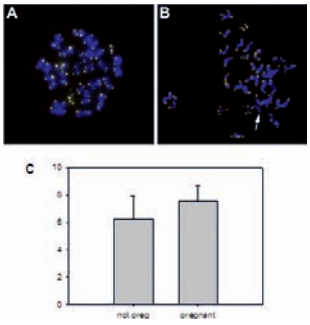

Figure 1. Telomere FISH for chromosomes of human oocytes. (*a*) Normal telomeres associated with successful pregnancy. (*b*) Chromosomes with shortened telomeres from a failed pregnancy. The arrow indicates telomere loss at the chromosome end. (*c*) Telomeres are shorter ($p < 0.005$) from failed pregnancy (preg), compared with pregnant women.

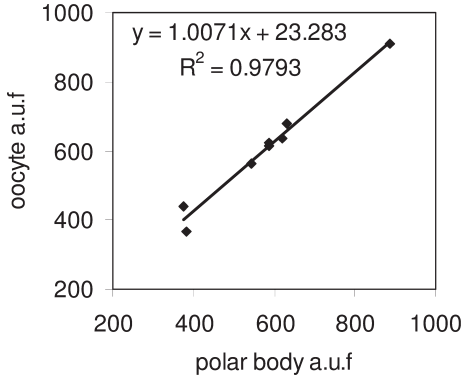

Figure 2. Prediction of average telomere length in egg chromosomes by analyzing the telomeres of their polar bodies. a.u.f., arbitrary unit of fluorescence. Data represent 8 pairs of oocytes with their polar bodies from 6 patients of different age groups.

among women of advanced reproductive age [41]. Ethical constraints limit production of human embryos strictly for the invasive studies needed to answer this question, but a study of sister eggs, aspirated during clinical in vitro fertilization (IVF) procedures, found their telomere length predictive of the developmental potential of their sib oocytes. The maximum (19.3 ± 3.1 kb vs. 13.9 ± 3.28 kb, $p < 0.01$) and mean (7.5 ± 1.17 kb vs. 6.2 ± 1.69 kb) telomere lengths are longer, and the variation, measured by standard deviation, greater (4.4 ± 0.96 vs. 3.5 ± 1.12) in eggs from patients who become pregnant after IVF, compared with those who fail to become pregnant after IVF. No patients become pregnant if their mean telomere length is less than 6.32 kb (Fig. 1). No other clinical parameters, including patients' age, baseline follicle stimulating hormone (FSH) level, egg number, body mass index, ovarian stimulation protocol, number of previous IVF cycles, diagnosis, or embryo morphology distinguish the pregnant and non-pregnant groups as well as telomere length. Decreased egg telomere length portends poor reproductive outcome in infertile women undergoing IVF. Telomere length provides a better predictor of pregnancy outcome following IVF than patient age itself or other clinical parameters, even when telomere length is measured only in spare eggs. Telomere length in chromosomes from spare eggs correlates highly with that in their associated first polar bodies (R2 = 0.98) (Fig. 2), suggesting that future studies may be able to estimate with greater accuracy the telomere length of the actual embryo being transferred, rather than estimating this based on measurement of telomere length in a spare, sister oocyte [42].

Conclusion

Telomeres play crucial roles in meiosis and in aging, and the data reviewed here indicate they also likely play a crucial role in mediating the effects of aging on meiosis. Clarification of the molecular mechanisms of telomere regulation of early meiosis provides a promising avenue for research, with implications for understanding both normal development and human disease.

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