Chromosomal and cytoplasmic context determines predisposition to maternal age-related aneuploidy: brief overview and update on MCAK in mammalian oocytes

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Abstract
It has been known for more than half a century that the risk of conceiving a child with trisomy increases with advanced maternal age. However, the origin of the high susceptibility to nondisjunction of whole chromosomes and precocious separation of sister chromatids, leading to aneuploidy in aged oocytes and embryos derived from them, cannot be traced back to a single disturbance and mechanism. Instead, analysis of recombination patterns of meiotic chromosomes of spread oocytes from embryonal ovary, and of origins and exchange patterns of extra chromosomes in trisomies, as well as morphological and molecular studies of oocytes and somatic cells from young and aged females, show chromosome-specific risk patterns and cellular aberrations related to the chronological age of the female. In addition, analysis of the function of meiotic- and cell-cycle-regulating genes in oogenesis, and the study of the spindle and chromosomal status of maturing oocytes, suggest that several events contribute synergistically to errors in chromosome segregation in aged oocytes in a chromosome-specific fashion. For instance, loss of cohesion may differentially predispose chromosomes with distal or pericentromeric chiasmata to nondisjunction. Studies on expression in young and aged oocytes from human or model organisms, like the mouse, indicate that the presence and functionality/activity of gene products involved in cell-cycle regulation, spindle formation and organelle integrity may be altered in aged oocytes, thus contributing to a high risk of error in chromosome segregation in meiosis I and II. Genes that are often altered in aged mouse oocytes include MCAK (mitotic-centromere-associated protein), a microtubule depolymerase, and AURKB (Aurora kinase B), a protein of the chromosomal passenger complex that has many targets and can also phosphorylate and regulate MCAK localization and activity. Therefore we explored the role of MCAK in maturing mouse oocytes by immunofluorescence, overexpression of a MCAK-EGFP (enhanced green fluorescent protein) fusion protein, knockdown of MCAK by RNAi (RNA interference) and inhibition of AURKB. The observations suggest that MCAK is involved in spindle regulation, chromosome congression and cell-cycle control, and that reductions in mRNA and protein in a context of permissive SAC (spindle assembly checkpoint) predispose to aneuploidy. Failure to recruit MCAK to centromeres and low expression patterns, as well as disturbing alterations in regulation of enzyme localization and activity, e.g. due to alterations in activity of AURKB, may therefore contribute to maternal age-related rises in aneuploidy in mammalian oocytes.

Introduction
Over 70 years ago, Penrose [1] described a correlation between Down’s syndrome (trisomy 21) and maternal age. Since then analysis of the chromosomal status of donated human oocytes [2], polar body biopsies [3], and analysis of the incidence of chromosomal aberrations in embryos, spontaneous abortions, stillbirth and live births [4], all confirmed that the risk of errors in chromosome segregation increases with advanced maternal age. There is an ongoing debate on whether it is mainly maternal age in itself (chronological age of the female, ovary and oocyte) or the depletion of the ovary from primordial follicles and primary oocytes (physiological ageing) that determine risks for nondisjunction at oogenesis [5–7]. Furthermore, it is not known to what extent the individual chromosomal constitution, defined by the length, gene distribution and content, and presence and pattern of exchanges on each individual chromosome, contribute to high susceptibility to meiotic errors in an aged oocyte. Trisomy data and data from animal models all suggest that failure of chromosomes to recombine is a general risk factor [8]. Lastly, there are different hypotheses on constitutive and age-related alterations in the hormonal homoeostasis, and in oocytes and follicles, which are believed to correlate with increased failures in faithful and sequential segregation of homologues and sister chromatids at meiosis I and II in oocytes [9]. In

Key words: aneuploidy, maternal age, mitotic-centromere-associated protein (MCAK), oocyte.

Abbreviations used: AURKB, Aurora kinase B; EGFP, enhanced green fluorescent protein; MCAK, mitotic-centromere-associated protein; ROS, reactive oxygen species; SAC, spindle assembly checkpoint.

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Figure 1 | Stages of oogenesis that are susceptible to disturbances and events leading synergistically to high risks for errors in chromosome segregation in oocytes at advanced maternal age

(A) Events in the embryonic ovary, including presence of trisomic cell line of oogonia [10], failure to recombine and/or numbers and placement of exchanges on homologous chromosomes [8,11–13]. (B) Events during long meiotic arrest of dictyate stage-arrested oocyte in the primordial follicle post-birth, including accumulation of insult by environment, life style/nutrition and ROS, loss of cohesion during chronological ageing and reduction of follicle pool (physiological ageing) by continuous recruitment and atresia of follicles [5–7,16–22]. (C) Pre-ovulatory events in the growing oocyte or after resumption of maturation by changes in follicular development, oxygen supply and synthesis/degradation of RNAs and cell-cell signalling, causing changes in gene expression and proteins affecting spindle formation, cell-cycle control and sequential chromosome segregation [25,26,28–33], including activity and distribution of MCAK (D). MCAK is usually associated with spindle poles (labelled by anti-pericentrin antibody in red), and centromeres of homologous chromosomes (stained in blue) in prometaphase I and metaphase I. The image in (D) shows the distribution of MCAK-EGFP fusion protein (green) at centromeres, centrosomes and faintly on the spindle in a maturing mouse oocyte at late prometaphase I stage [47]. For further explanation, see the text.

the following review we will give a brief overview of stages of oocyte development and events influencing the context under which chromosomes become separated during the late stages of meiosis under a more or less risky cellular environment predisposing to nondisjunction.

Results and observations in human and animal models

Stages of oocyte development and origins of susceptibility to nondisjunction

Events in the embryonal ovary prior to birth

Preparation for oogenesis is already primed in the last mitotic oogonial divisions before oocytes actually enter meiosis in the embryonic ovary. One hypothesis suggests that a pool of trisomic oogonia reside in the embryonic ovary that gives rise to oocytes with incomplete and aberrant chromosome synapsis and recombination patterns. The latter are at high risk for producing a trisomic secondary oocyte [10]. Under the condition that such oocytes are developing slowly, survive in primordial follicles and become recruited for resumption of maturation only late in the reproductive period according to a production line [5] they could contribute to increases in numbers of aneuploid oocytes in older females. In normal diploid oocytes the absence of exchanges or placement, and the numbers of crossing overs that are established in the embryonic ovary, may also relate to the relative risk of each individual chromosome undergoing nondisjunction or facilitating normal distribution after resumption of maturation in the cytoplasm of an aged oocyte [11–13]. This means that there is a link between early and late events in oogenesis. A summary of these events is shown in Figure 1(A).

Dictyate stage arrest

Oocytes are not metabolically quiescent [14]. The greatest change in gene expression takes place at the transition
from primordial to primary follicle, i.e. when follicles and oocytes are recruited from the resting to the growing pool. Organelles and molecules within the oocyte and the somatic compartment can be exposed to noxious influences in dependence on chronological age, life-style and environment, as well as intrinsic ageing due to damage by ROS (reactive oxygen species) from metabolism. Therefore the long meiotic arrest of oocytes that resume maturation only late in the reproductive life of a female may be responsible for an accumulation of aberrations that ultimately contribute to compromising normal chromosome segregation [15]. For instance, mitochondria and organelles in somatic cells and oocytes of aged ovaries are morphologically altered [16,17] and this can lead to dysfunction, reduced capacity to supply cells with ATP, and regulate calcium homoeostasis and enzyme activities (e.g. kinases) or support synthesis of RNAs and proteins (discussed in [18]). Accumulation of adducts from exposure to ROS, such as AGEs (advanced glycation end-products), and damage to lipid membranes in mitochondria and other cell organelles etc. can contribute to a decrease in the ability of the aged oocyte to form a functional spindle and to control chromosome segregation [17–19]. Homologues in bivalents are physically held together by cohesion complexes at sister chromatid arms and centromeres, and complexes of cohesion proteins are already recruited at the beginning of meiosis. Transient decay and proteolysis of meiotic cohesins during a long arrest-phase could contribute to precocious chiasma resolution when cohesins do not become replaced, and thus susceptibility to random segregation may become increased in functional univalents once oocytes resume maturation [20,21]. In addition, the loss of cohesion proteins might affect chromatin conformation and gene expression in the aged oocyte [22] and thus contribute to aberrations in the normal expression patterns that are required to control chromosome segregation and oocyte quality. A summary of these events is shown in Figure 1(B).

**Oocyte growth phase**

Oocytes acquire high nuclear and cytoplasmic developmental potential during a growth phase which is dependent on the bi-directional communication between the oocyte and the somatic cells in the follicle and the regulation of the hormonal environment [23,24]. Gene expression in both the oocyte and the somatic cell compartment are fine-tuned by auto- and para-crine signalling. When the metabolism of the granulosa cells, producing molecules such as lactate, pyruvate, amino acids and cholesterol, becomes altered, this can profoundly affect intrinsic regulation of gene expression [24] and establishment of cellular micro-domains supporting spindle formation and chromatin conformation in the oocyte [18]. Disturbances in junctional contacts e.g. by homo- and hetero-oligomeric gap junctions between the oocyte and the follicle, can be responsible for changes in the relative abundance of metabolites, such as lactate or pyruvate, that are essential for normal spindle formation and sequential segregation of homologues and chromatin [25,26]. cAMP and cGMP, factors maintaining oocytes in meiotic arrest [27], and also the relative abundance of proteins, for instance, those protecting from oxidative stress within oocytes or follicles [28], may be of relevance. Fully grown oocytes with high developmental potential that reside in large antral follicles and characteristically become transcriptionally quiescent and possess condensed chromatin surrounding the nucleus are known as SN oocytes. SN oocytes of mouse that do not become ovulated in stimulated cycles and are induced to mature in vitro are highly susceptible to errors in chromosome segregation [29]. It is not known whether altered cyclic and hormonal homoeostasis in a female of reproductive age with depleted pools affect the developmental programme subtly, such that follicles and oocytes of suboptimal quality can survive, become dominant and be triggered to resume maturation too early or too late relative to the intrinsic changes in chromatin configuration that usually precede maturation. A summary of these events is shown in Figure 1(C).

**Oocyte maturation and spindle formation**

The ovary is highly vascularized and increased vascularization of follicles and increased blood flow may contribute to supplying somatic cells and oocytes of the growing and maturing follicle with sufficient oxygen. It has been suggested that reduced vascularization of follicles in depleted ovaries of older females contributes to oxidative stress and compromises activities of granulosa cells and spindle formation in oocytes [30]. Donated and spare human oocytes from older patients tend to have aberrant spindles and fail to align chromosomes properly at metaphase II [31,32]. GV-stage oocytes that are isolated from the ovary of older females in natural oestrous cycle, and come from some strains of mice that exhibit maternal-age-related rises in aneuploidy, undergo anaphase I faster compared with those from young females, similar to mice deficient in spindle checkpoint proteins [20,33–35]. Such aged oocytes are still capable of responding to major disturbances in spindle formation when exposed to high concentrations of microtubule-depolymerizing chemicals. Therefore it has been suggested that there is a more permissive cell-cycle control in addition to the other changes, including spindle aberrations in aged oocytes, and this contributes synergistically to high susceptibility to meiotic errors [20]. Whether loss of cell-cycle control is a general feature of aged oocytes has been questioned since oocytes from superovulated aged female mice of some strains do not mature faster, although errors in chromosome segregation are increased with age [36]. There is evidence that in mice, adult-onset caloric restriction maintains function of the female reproductive axis into advanced ages, possibly postponing age-related aneuploidy [37]. Therefore there may be a link between life-style, metabolism, follicle health and oocyte susceptibility to meiotic error that deserves further research. A summary of these events is shown in Figure 1(C).

**Differences in expression patterns and susceptibility to nondisjunction**

With the advent of assisted reproduction and the possibility of quantifying cellular components such as ATP, mRNAs
or proteins in individual or few donated human oocytes or oocytes from model organisms from bovine to rodent, much information has been gained on relative differences between young and aged oocytes. Collectively, transcriptome data suggest that the abundance of mRNA varies between young and aged oocytes, although there is only a limited extent to which specific mRNAs are consistently lower or higher in aged compared with young oocytes [38–41]. Most studies have focused on mRNA patterns in metaphase II oocytes. These may not reflect the relative abundance of proteins at maturation since many messages are recruited and/or degraded during oocyte maturation in a well-orchestrated cell-cycle-dependent fashion [42,43]. However, decreases or increases in abundance of mRNAs at metaphase II are likely to reflect disturbances in mRNA recruitment, polyadenylation, translation and degradation [40]. Most studies consistently find differences in the relative abundance of mRNAs of cytoskeletal and spindle components, such as tubulins or motor proteins, involved in cell-cycle control, e.g. proteins involved in the SAC (spindle assembly checkpoint), monitoring attachment of chromosomes to spindle fibres, and oxidative defence. In addition, it appears that there are differences in the abundance of mRNAs that affect mitochondrial function [39], protein degradation [40] and of maternal products needed in early embryogenesis before zygotic gene activation [38]. Several studies modelling alterations in gene expression in spindle formation, cell-cycle progression and chromosome congression, which are also affected by ageing, support the view that the alterations at the transcriptome level critically affect chromosome segregation [34,40]. Among the genes whose transcripts were significantly altered in aged compared with young metaphase II mouse oocytes are those coding for MCAK (mitotic-centromere-associated kinesin) and the serine/threonine kinase, AURKB (Aurora kinase B) [40]. Therefore we explored the function and consequences of altered abundance or activity of these factors in maturing mouse oocytes.

Consequences of altered expression of MCAK for predisposition to nondisjunction

MCAK belongs to the kinesin-13 family of proteins with a centrally located motor domain, which possesses microtubule depolymerizing activity, and has been implicated in correction of false attachments of chromosomes (e.g. merotelic attachments) to spindle poles and in regulation of microtubule dynamics in mitotic cells [44,45]. In comparisons between the transcriptome of young and aged oocytes of the mouse, MCAK mRNA was one of the mRNAs that was expressed at a level significantly lower in aged compared with young oocytes [40]. In addition, a kinase that has been shown to influence the microtubule depolymerase activity of MCAK, AURKB, a component of the CPC (chromosomal passenger complex), which is involved in cell-cycle control, regulation of sister chromatid cohesion and in cytokinesis [44–46], was also altered in expression [40]. Using immunofluorescence and expression of an MCAK–EGFP (enhanced green fluorescent protein) fusion protein in maturing mouse oocytes we could show that MCAK associates with spindle poles, centromeres and chromosome arms and appears enriched at chiasmata at meiosis I, prior to anaphase I and telophase I [47] (Figure 1D). At anaphase I MCAK becomes recruited to the central spindle and midbody, and at prometaphase II and metaphase II again resides at the centromeres and spindle poles [47]. Knockdown of MCAK causes a delay in chromosome congression at meiosis I and a meiotic arrest that can be overcome by knockdown of Mad2 suggesting an involvement in the release from the SAC. Progression to meiosis II in Mad2- and MCAK-depleted oocytes causes formation of aberrant spindles, failures in chromosome congression in a large number of oocytes, and increases in hypoploidy, but not hyperploidy [47]. Inhibition of AURKB from the beginning of resumption of maturation by low concentrations of ZM447439 significantly blocks cytokinesis [48]. However, not all oocytes undergoing germinal vesicle breakdown that fail to emit a polar body contain bivalents. Rather only a fraction is polyploid with twice the number of dyads; in the latter anaphase I presumably progressed in the absence of cytokinesis. A large percentage of the ZM447439-exposed oocytes contain bivalents, as well as dyads, suggesting that inactivation of AURKB blocks loss of chromosome cohesion [48]. This may relate to disturbances in AURKB-mediated association of separate with chromosomes, similar to findings in mitotic cells [49]. Inhibition of AURKB at late metaphase I does not affect anaphase I progression and cytokinesis. It also does not inhibit the tri-methylation of Lys9 of histone H3 at centromeres, as is observed in meiosis I oocytes exposed continuously to the inhibitor [48]. However, late exposure to the inhibitor causes lagging of chromosomes at the anaphase I transition, which might relate to interference with MCAK localization or activity by the inhibition of AURKB [47]. In conclusion, the observations suggest that alterations in the abundance and activity of MCAK and AURKB may contribute to predisposition to errors in chromosome segregation in aged oocytes. This is particularly true when checkpoint controls are also permissive and there is already some loss of chromosome cohesion and reductions in proteins such as shugoshin 2 that contribute to cell-cycle-specific recruitment of MCAK to centromeres, as shown in male meiosis [50].

Conclusions

It appears that multiple events during oocyte maturation contribute to differential predisposition of individual chromosomes to errors in chromosome segregation involving whole chromosomes or sister chromatids at meiosis I or II in the context of an ‘aged ooplasm’. Presence, localization and numbers of exchanges as a result of early meiotic events predispose chromosomes differentially to mis-segregation, depending on age. The long meiotic arrest possibly affects chromosome cohesion and the integrity and functionality of cellular components within the oocyte and the follicular environment. The depletion of the follicle pool has a profound effect on hormonal homeostasis. Within this ‘aged context’,
altered expression during oocyte growth and maturation, for instance, of critical components of the cell-cycle control and spindle processes, and the deregulated recruitment and/or degradation of mRNAs and proteins, may ultimately act together to predispose oocytes to errors in chromosome segregation. We present an example showing that altered expression or activity of MCAK and AURKB interfere with the alignment and segregation of chromosomes in maturing oocytes that contribute to loss of fidelity of chromosome segregation, particularly in the context of permissive checkpoints and conditions mimicking the situation in aged oocytes.

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**References**

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