Ceramide and mitochondrial function in aging oocytes: juggling a new hypothesis and old players

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Abstract

Maternal aging adversely affects oocyte quality (function and developmental potential) and consequently lowers pregnancy rates while increasing spontaneous abortions. Substantial evidence, especially from egg donation studies, implicates the decreased quality of an aging oocyte as a major factor in the etiology of female infertility. Nevertheless, the cellular and molecular mechanisms responsible for the decreased oocyte quality with advanced maternal aging are not fully characterized. Herein we present information in the published literature and our own data to support the hypothesis that during aging induced decreases in mitochondrial ceramide levels and associated alterations in mitochondrial structure and function are prominent elements contributing to reduced oocyte quality. Hence, by examining the molecular determinants that underlie impairments in oocyte mitochondria, we expect to sieve to a better understanding of the mechanistic anatomy of oocyte aging.

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Maternal age and fertility

Female fecundity (the ability to produce offspring) reaches its peak at 30 years of age, then declines abruptly and is lost by ~50 years of age at the onset of menopause (Faddy 2000, Gougeon 2005). This phenomenon is due to a combination of factors, but in general, subfertility in older women is primarily related to: poor quality of aging oocytes, reflecting chromosomal, morphological, and functional abnormalities (Beemsterboer *et al.* 2006, Hunt & Hassold 2008, Broekmans *et al.* 2009, Perheentupa & Huhtaniemi 2009); decreased ovarian reserve, marked by fewer oocytes (Richardson *et al.* 1987, Ames *et al.* 1995, Broekmans *et al.* 2009, Perheentupa & Huhtaniemi 2009); and an altered hormonal environment resulting in ovulatory dysfunction (Broekmans *et al.* 2009). Only about 5% of women with diminished ovarian reserve presently achieve pregnancy, despite use of ovulation inducing agents (Gougeon 2005, Braveman 2006, Habbema *et al.* 2009). In the last 30 years a worldwide trend by women to delay childbearing until onset of the decline in fecundity (late 30s and 40s) has increased the risk of infertility (Ottolenghi *et al.* 2004). Assisted reproductive technologies (ART) have compensated for the decreased natural fertility, but only to a limited extent (Braveman 2006, Habbema *et al.* 2009), leaving many couples

childless despite prolonged and demanding therapies for infertility.

Owing to lack of knowledge, the mechanisms behind the gradual decline of the follicle pool and the reduced oocyte quality are far from being fully understood. According to Hunt & Hassold (2008), female fertility is influenced by a complex series of events that occur at any of the three critical stages of oocyte development, identified as: the initiation of meiosis in the fetal gonad; formation of primordial follicles during late gestation and the perinatal period; and oocyte growth and maturation taking place in the adult. Thus, it seems increasingly likely that age-related changes affecting the quality of the oocyte reflect a complex interplay of events involving both nuclear and cytoplasmic components.

Since age is highly negatively correlated with oocyte quality, advance maternal age is the most prominent impediment to a successful assisted reproductive program (Janny & Menezo 1996, Hunt & Hassold 2008, Habbema *et al.* 2009). The evidence for impact of age is apparent in the increased percentages of embryo fragmentation during early cleavage stages in embryos from older women (Jurisicova *et al.* 2006), and by an elevated cell death index in blastocysts from aged mice (Keefe *et al.* 1995, Acton *et al.* 2004).

A mathematical model of death rates in human preimplantation embryos suggests that the factors

predisposing an embryo to developmental arrest are determined at the zygote stage or earlier (Hardy *et al.* 2001). The initial stages of embryo development are sustained by maternally provided transcripts and proteins accumulated in the oocyte during oogenesis (Schultz & Heyner 1992, Minami *et al.* 2007, Tang *et al.* 2007). These factors are crucial for early developmental events in embryos, including axis formation, cell fate determination, and activation of the embryonic genome. Furthermore, postfertilization, the oocyte cytoplasm is solely responsible for remodeling of the incorporated paternal chromatin and plays a role in epigenetic modifications of the newly formed embryonic nucleus (Torres-Padilla *et al.* 2006, Yoshida *et al.* 2007). Since factors from the oocyte cytoplasm and products of its gene expression control many processes central to the early development of the whole organism, it is not surprising, therefore, that a decline in oocyte quality has a profound impact on the developmental competence of the embryo and contributes to the high incidence of embryonic wastage observed during IVF procedures in ART clinics (Jurisicova & Acton 2004).

Mitochondria in germ cells

Mitochondria are the most prominent cell organelles in oocytes, and they represent one of the most important maternal contributions to early embryogenesis (Van Blerkom 2004, 2011, Van Blerkom *et al.* 2006, Dumollard *et al.* 2007, Eichenlaub-Ritter *et al.* 2011). Owing to their role in the production of cellular energy as well as in the control of cell death, these organelles are at the center of death and life decisions in most cells, including the oocyte (Perez *et al.* 2000, Wang 2001, Danial & Korsmeyer 2004). Moreover, mitochondrial activity appears to be essential for normal spindle formation and chromosome segregation (Eichenlaub-Ritter *et al.* 2004). However, compared with the other cell types in the body, germ cells' mitochondria are unique. For example, in comparison to those in somatic cells, oocyte mitochondria have been described as morphologically primitive or immature, due to the fact that they are smaller and possess less complex internal structures (Dumollard *et al.* 2007).

The number of cristae in a mitochondrion has been directly correlated to the level of ATP production (Jansen & de Boer 1998). As such, the germ cell mitochondria with their limited number of cristae and denser matrices have diminished ability to generate ATP. This has been demonstrated in human oocytes by Van Blerkom *et al.* (1995). Recently, several studies on the metabolic activity of oocytes and early embryos concluded that, although their mitochondria have low activity, they are constitutively active; and that maintenance of that low-level activity is necessary and sufficient for ongoing development (Cummins 2004a,b, Van Blerkom 2004, 2011, Van Blerkom *et al.* 2006, Dumollard *et al.* 2007).

Nevertheless, in aged mouse oocytes we have recently found that the number of mitochondrial cristae is even lower compared with younger oocytes, which may compromise development. It should be noted that mitochondria in a zygote are maternally inherited, the few paternal mitochondria that enter the oocyte during fertilization are targeted to ubiquitin-dependent proteolysis (Giles *et al.* 1980, Cummins 2000, 2002, Sutovsky *et al.* 2000), or diluted out during sequential cleavage (Cummins 2000). Since the mitochondria in oocytes have a limited number of replication cycles before the blastocyst stage (McConnell & Petrie 2004), the developing embryo is inevitably designed to depend entirely on the population of mitochondria present at the time of ovulation. Moreover, in contrast to most cells, oocytes and cleavage stage embryos rely on mitochondrial ATP alone since glycolysis is pretty much switched off.

It is reported that deletions in mitochondrial DNA (mtDNA) are significantly higher in oocytes than in embryos, strongly suggesting that mtDNA integrity plays a role in determining the fertilizability of oocytes (Brenner *et al.* 1998, Barritt *et al.* 1999). Evidence that mitochondria directly determine the fate and quality of oocytes and embryos has been demonstrated by us and others in studies where microinjection of pure mitochondria or mitochondria-enriched cytoplasts into mouse oocytes reduced apoptotic rates (Perez *et al.* 2000) and increased ATP production (Van Blerkom *et al.* 1998). Additional support is provided by the fact that ooplasm transfer to poor quality recipient oocytes in ART clinics decreased oocyte and embryo fragmentation rates, improved embryo development and led to the birth of at least 30 children worldwide (Cohen *et al.* 1998).

Therefore, as in any other cell, it is expected that adequate generation of energy within oocytes will be of primary significance for processes such as oocyte maturation, chromosome segregation, and developmental capacity (Eichenlaub-Ritter *et al.* 2010). Any changes in activity of mitochondria (e.g. redox homeostasis) and in their morphology, as those seen during aging, are thus expected to be crucial for fertility. Age-associated mitochondrial damage is anticipated to be most critical for stages with higher energy demands such as oocyte maturation and early embryo development right up to the time of zygotic gene activation.

Mitochondria and oocyte aging

Mitochondrial dysfunction has been implicated in both general body aging (Ames *et al.* 1995, Sastre *et al.* 2002) and aging of female reproductive tissues (Janny & Menezo 1996, Jansen & de Boer 1998, Kirkwood 1998, Ruman *et al.* 2003, Tarlatzis & Zepiridis 2003, Ottolenghi *et al.* 2004, Gougeon 2005). Our most recent observations demonstrate that, both function and morphology of mitochondria are impaired in oocytes from older mice (GI Perez & LL Kujjo 2011, unpublished

observation). And hence, considering the importance of mitochondrial function in the oocyte, it is not surprising that oocyte quality decreases with aging (Ruman *et al.* 2003, Tarlatzis & Zepiridis 2003, Ottolenghi *et al.* 2004, Gougeon 2005). This in turn may translate into a fall in the success rates of IVF cases, partially due to increases in oocyte mitochondrial defects. Evidence for this is further provided by the correlated increase of maternal age with: increase in the rate of DNA mutations in oocyte mitochondria (Keefe *et al.* 1995); decrease in mitochondrial metabolic activity (Wilding *et al.* 2001); inefficiency in mitochondrial ATP production (Van Blerkom *et al.* 1995); and changes in mitochondrial calcium homeostasis (Van Blerkom 2011).

In elegant experiments using mouse oocytes as a model of mitochondrial dysfunction, Thouas *et al.* (2004, 2005, 2006) demonstrated that sensitivity to mitochondrial injury is developmentally regulated and increases with age. For example, when young oocytes were subjected to low degrees of mitochondrial injury by exposing them to photosensitization, further development to blastocyst *in vitro* was not affected. By comparison, *in vitro* development of preimplantation embryos from aged oocytes was more sensitive to equivalent mitochondrial damage. The authors concluded that age-related mitochondrial energy deficiency might account for the differences observed, and suggested a possible direct linkage of delayed developmental effects to mitochondrial deficiencies caused by advanced aging (Thouas *et al.* 2005). These data are in agreement with those from human oocytes, showing that decreases in activity of mitochondria derived from oocytes of older women are associated with lower embryonic development and low pregnancy rates compared with oocytes obtained from younger women (Van Blerkom *et al.* 1995).

In addition to generalized aging of oocyte cytoplasm, increase in dysfunctions of mitochondria with advanced age might also contribute to nuclear genome instability in oocytes, as reported for other cells (Veatch *et al.* 2009). As such, age-related increases in aneuploidy in oocytes might be direct or indirect consequences of decreased mitochondrial activity. For example as described in recent publications (Cukurcam *et al.* 2007, Eichenlaub-Ritter *et al.* 2010), occurrence of spindle aberrations attributed to insufficient energy supply, and/or shifts in redox regulation, might influence enzyme activities and cause loss of chromosome cohesion, or chromosome integrity and stability, especially during early embryogenesis. Thus, there is sufficient evidence that with advanced age mitochondrial pathophysiology contributes to decreased female fertility. However, the causative factors leading to aging-related changes in mitochondrial structure and function for the most part remain unknown.

Although age-related mutations in oocyte mtDNA affect oocyte quality, it is anticipated that those effects would be subtle, since only a fraction of the numerous

mitochondria present in the germ cells would be affected (Eichenlaub-Ritter *et al.* 2011). On the other hand, we hypothesize that age-related alterations in mitochondrial lipids or any other global structural mitochondrial damage are expected to have a more pronounced impact on overall mitochondrial activity because they affect all mitochondria.

Ceramide and oocyte aging

Considering the preceding discussion on the impact of mitochondria on oocyte quality, we hypothesize that: during aging, a dysregulation in the intracellular transport and/or synthesis of ceramide (a bioactive lipid) occurs, and that this prevents ceramide from reaching the normal levels in mitochondria. This lipid imbalance leads to less functional mitochondria and a negative effect on oocyte quality.

Ceramide is the basic structural component of many lipids known as sphingolipids. In this central role, ceramide is utilized for the synthesis of other bioactive sphingolipids, including sphingosine-1-phosphate, and sphingosine (SPH), and the glycosphingolipids hexosylceramide and lactosylceramide (Hannun & Obeid 2011).

Sphingolipids, specifically ceramide, as well as mitochondria, are implicated in both development and aging at the organismal (Cutler & Mattson 2001) and the tissue level (Lightle *et al.* 2000). Currently, ~200 distinct ceramide species are known to exist in mammalian cells (Hannun & Obeid 2011). These ceramides are synthesized in a combinatorial fashion, with distinct enzymes responsible for the specific modifications (Gault *et al.* 2010). As shown by Hannun & Obeid (2008) ceramides can be generated through the action of sphingomyelinase (SMase) or via the *de novo* synthetic pathway through the action of ceramide synthases (CerSs). Ceramides generated via SM hydrolysis can be further hydrolyzed by ceramidases (CDases) to form SPH, which can then be reacylated via the action of CerS (also known as LASS) to regenerate ceramide species. These multiple pathways of ceramide generation led to the hypothesis that individual ceramide molecular species are regulated by specific biochemical pathways in distinct subcellular compartments and execute distinct functions (Hannun & Obeid 2011, Novgorodov *et al.* 2011).

Mitochondria are important intracellular compartments of sphingolipid metabolism (Novgorodov & Gudz 2009), and several sphingolipid-metabolizing enzymes were found to be associated with mitochondria; so far the list includes neutral ceramidase (El Bawab *et al.* 2000), novel neutral SMase (Wu *et al.* 2010, Clarke *et al.* 2011), and (dihydro) CerS, a key enzyme in *de novo* ceramide synthesis (Bionda *et al.* 2004, Yu *et al.* 2007). The existence of these compartment-specific pathways clearly suggests high specialization of these pathways, which in turn suggests specific mechanisms of

regulation and, equally as likely, distinct functions and mechanisms of action of their lipid products.

Ceramide signaling involves a complex network of molecules and subcellular organelles all implicated in a range of cellular processes such as necrosis (Hetz *et al.* 2002), survival and proliferation (Adam *et al.* 2002), differentiation (Okazaki *et al.* 1990), and aging (Venable *et al.* 2006). The involvement of sphingolipids in aging is not new; after all, there is considerable evidence linking sphingolipid genes with lifespan. For example, deletion of the longevity assurance gene (*LAG1*), one of the first genes implicated in yeast aging (D'Mello *et al.* 1994) was shown to extend lifespan. Interestingly, this gene was subsequently discovered to encode a CerS (Guillas *et al.* 2001, Schorling *et al.* 2001). Furthermore, some pathways of sphingolipid metabolism have been shown to play an important role in determining *Drosophila* lifespan (Rao *et al.* 2007, Yang *et al.* 2010). Recently, Yang *et al.* (2010) reported that the *Drosophila* alkaline ceramidase (catalyzes breakdown of ceramide into SPH and fatty acids) plays an important role in *Drosophila* development and longevity; apparently, the mutation of alkaline ceramidase significantly increases mean and maximum lifespan. Finally, mutations of the ceramide transfer protein (CERT) in flies caused enhanced oxidative damage and dramatically reduced their lifespans (Rao *et al.* 2007). By virtue of its function, CERT regulates cellular ceramide levels by transporting ceramide from the endoplasmic reticulum (ER) to the Golgi where it is used specifically for SM synthesis (Hanada *et al.* 2007). Taken together, these studies support the role of sphingolipids in determining lifespan, and as such are important regulators of the aging process.

It is perhaps not surprising that we recently identified ceramide as one of the key molecules signaling the accelerated incidence of apoptosis in oocytes of aged female mice (Perez *et al.* 2005). This finding highlights a novel role for the intercellular trafficking of ceramide as a key step in this process of accelerated oocyte death. Therefore, we believe that this novel role of ceramide is associated with the aging-related decline of ovarian function (Richardson *et al.* 1987, Faddy *et al.* 1992). Our conclusion is supported by the fact that ceramide levels have been observed to increase during the years immediately preceding menopause (Diatlovitskaia *et al.* 1995), a phase in a woman's life when the endowed pool of germ cells (oocytes) has been nearly exhausted (Richardson *et al.* 1987).

We demonstrated that in aged mice the levels of ceramide increase in the cumulus cells but decrease in the oocyte. This chronic depletion of oocyte ceramide is a prerequisite that sets up the oocyte to become sensitive to ceramide spikes released by the cumulus cells and translocated via gap junction-dependent communication (Perez *et al.* 2005). So, in the aging oocyte we have delineated two patterns of ceramide

concentrations: one dependent on what we call 'pre-cumulus release', characterized by lower levels of ceramide, such as the ones we previously reported (Perez *et al.* 2005); this occurs around 9 months of age in ICR mice. The second concentration pattern is sustained by 'post-cumulus release'; it occurs somewhere after 12 months of age, being characterized by high levels of ceramide, and is responsible for accelerated oocyte death with age. Considering the fact that the accelerated rate of oocyte depletion from the human ovaries coincides with the time when ceramide levels increase, it is logical to speculate that the events we observed in the aging mice also underlie similar mechanisms in aging human oocytes. Nevertheless, so far no data have been published to indicate any association between human maternal age and the lipid content of oocytes.

In further studies we also discovered that signaling of survival and death pathways in the oocyte are influenced by the spatial locations of ceramide pools (Perez *et al.* 2005). In oocytes from young mice, exogenous ceramide rapidly accumulates in mitochondria and prevents oocyte apoptosis. In contrast, in oocytes from aged mice, exogenous ceramide does not reach the mitochondria but accumulates in the cytoplasm (possibly in the ER) and triggers cell death (Fig. 1; GI Perez 2011, unpublished observation). The emerging view is that distinct ceramides in independent compartments serve as local centers of sphingolipid metabolism (Hannun & Obeid 2011). In turn, protein-mediated transfer and vesicular transport of sphingolipids then serve to connect these various centers of activity. It is quite unlikely that ceramide formed in the lysosomes by the action of acid SMase would exert the same specific effects as ceramide formed in the mitochondria or the plasma membrane by the regulation of CDases or neutral SMase. Therefore, mechanistic studies on ceramide function and regulation should focus on specific pathways of formation. Three recent reviews have highlighted the physicochemical dynamics of ceramide signaling, and stressed the need to pinpoint the specific membrane ceramides associate with (van Blitterswijk *et al.* 2001, Hannun & Obeid 2008, 2011). Our laboratory is currently monitoring and localizing the cytoplasmic pools of ceramide in oocytes and their relationship to age, oocyte quality, and developmental potential. Currently, it is difficult to predict whether an observed change in ceramide will lead to apoptosis or ER stress or senescence. According to the new hypothesis, defining the pathway regulating ceramide, where in the cell this occurs, and what specific species of ceramide are involved will result in a much more robust understanding of that specific pathway of cell regulation and in predicting its function.

Ceramide transport protein and aging

In the cell, several organelles are responsible for the synthesis of ceramide (see recent review by

Figure 1 Exogenous ceramide localizes primarily to the mitochondria in young oocytes but not in old oocytes. Colocalization of ceramide and mitochondria in young oocytes is depicted in the merged image (yellow color). Methods: mature oocytes were cultured in HTF medium supplemented with 0.5% BSA at 37 °C in the presence of fluorescent ceramide (60 μ M) for 1 h. After 30 min, mitotracker red was added (250 nM) for an additional 30 min. Oocytes were washed in excess of media and visualized live using deconvolution microscopy.

Hannun & Obeid (2011)). The pathway for the *de novo* synthesis of ceramide is initiated in the ER and continues in the Golgi. In addition, ceramide synthesis also takes place in the plasma membrane, and most probably in the mitochondria as well. Some ceramide molecules are highly hydrophobic and therefore, they need to be generated in close proximity to their site of action by the activity of enzymes located near by. In the case where transport between organelles is required, specific sphingolipid-binding transporter proteins are involved (Futerman & Riezman 2005, Hannun & Obeid 2011). To date, CERT is the only known specific ceramide transport protein. This protein was identified by Hanada (2006) and works in the non-vesicular transport of ceramide from the ER to the Golgi apparatus. CERT is also known as the Goodpasture antigen-binding protein (Hanada *et al.* 2003, 2007, Hanada 2010, Mencarelli *et al.* 2010).

The biochemical and physicochemical aspects of CERT function have been extensively studied by Hanada *et al.* (Hanada *et al.* 2003, 2007, Kumagai *et al.* 2005, Hanada 2006, Kawano *et al.* 2006) by *in vitro* models. But, it was not until recently that the function of CERT was studied in a relevant physiological model, when Rao *et al.* (2007) demonstrated that CERT function is essential for normal oxidative stress response and lifespan in *Drosophila*. Recently, the same group (Wang *et al.* 2009) demonstrated that CERT is essential for mouse development and embryonic survival. Interestingly, these authors also found that CERT is critical for mitochondrial integrity.

Of particular interest is the fact that the majority of the phenotypic changes in the CERT mutant flies have striking similarities with the changes we and others have observed in the aged (human and mouse) oocyte. Specifically, the mutant flies: 1) are susceptible to reactive oxygen species; 2) develop metabolic imbalance including decreased ATP levels; 3) exhibit a 70%

decrease in ceramide levels; and 4) die early in life. The findings in aged oocytes include: higher susceptibility to reactive oxygen species (ROS; Thouas *et al.* 2005); reduced ATP levels (Van Blerkom *et al.* 1995); and lowered levels of total ceramide (Perez *et al.* 2005) that precede an increased rate of apoptosis. Since there are striking similarities between changes observed in the mutant flies lacking CERT and the changes observed in the aging oocytes, we hypothesized that in the aged oocyte CERT is downregulated, with consequential compromise of the oocyte developmental potential. A prediction that we recently confirmed (GI Perez & LL Kujjo 2011, unpublished observation).

Interestingly, it has been recently shown that the intermembrane ceramide transport catalyzed by CERT is sensitive to fluidity of the lipid environment (Tuuf *et al.* 2011). Therefore, if ceramide is in a tightly packed environment, the CERT transfer activity is markedly reduced. On the other hand, the ceramides in more fluid membranes are more available for CERT-mediated transfer. CERT also favors membranes that contain phosphatidylinositol 4-monophosphate (PI4P; Tuuf *et al.* 2011). Of interest is that, among the subcellular changes associated with advanced age can be discerned increases in membrane rigidity (Pepe 2005) and decreases in PI4P content of organelle membranes such as Golgi and mitochondria (Tran *et al.* 1993). Therefore, it would not be totally surprising to find that the transport of ceramide might also be impaired in aged oocytes.

Hypothetical model of ceramide trafficking in oocytes

The current literature and our data (published and unpublished) provide support for the involvement of ceramide in the age-dependent changes of oocyte mitochondrial structure and/or function, and led us to our hypothetical model (Fig. 2). The model posits that

Figure 2 Hypothetical model of ceramide trafficking in oocytes. We hypothesize that poor quality of old oocytes results from ceramide accumulating in the ER as a consequence of disabled ceramide transport to the Golgi, due to downregulation of CERT during aging. This indirectly reduces mitochondrial levels of ceramide, because the cell fails to provide sufficient sphingosine for the synthesis of mitochondrial ceramide. SPH, sphingosine; SM, sphingomyelin; C16, C16-ceramide; CER, ceramide; CERT, ceramide transport protein.

downregulation of CERT with advanced aging (GI Perez & LL Kujjo 2011, unpublished observation) is the modular response responsible for the changes in the organellar levels of ceramide.

We propose that under normal conditions ceramide (C16-ceramide) originating from the cumulus cells reaches the lysosomal compartment of an oocyte where it is hydrolyzed to SPH. SPH is then transported preferentially to the ER, but some little trafficking is directed to the mitochondria. In the ER and mitochondria (MITO) *de novo* synthesis of ceramide (CER) occurs. The ceramide formed in the ER is transported to the Golgi, which is the site of synthesis of SM. The transport of CER to the Golgi occurs through the action of the transfer protein CERT that specifically delivers CER for SM synthesis. SM is delivered to the plasma membrane where it is metabolized to other bioactive lipids, among those are ceramide and SPH. Under normal circumstances, in young oocytes the described pathways work just fine. But, in the old oocyte downregulation of CERT directly causes accumulation of ceramide in the ER that induces ER stress and apoptosis. Indirectly, downregulation of CERT reduces mitochondrial levels of ceramide, because the cell fails to provide sufficient SPH, the ceramide precursor, consequently impairing MITO function and morphology. As to which response occurs first or is more important between the ER stress and the mitochondrial alterations, still remains to be demonstrated.

Despite the complex biochemistry of ceramide, our endeavor is to further understand the cellular and molecular mechanisms that maintain physiological levels of this sphingolipid *in vivo* in oocytes, and implications for the aging process itself. At least two possibilities may account for ceramide increases in mitochondria. First, ceramide formed in the ER can be transferred to MITO via catalyzed exchanges of ER–MITO membrane contacts (Novgorodov *et al.* 2011). In contrast, MITO could

represent a specialized compartment of sphingolipid metabolism with their own subset of biosynthetic and degradative enzymes. A conventional view suggests that ceramide content is balanced by enzyme activities, involving both ceramide production (CerSs and SMases) and ceramide degradation (CDases; Hannun & Obeid 2008). Intriguingly, purified neutral CDase (NCDase) catalyzes both the hydrolysis and synthesis of ceramide (Tani *et al.* 2000, El Bawab *et al.* 2001). Novgorodov *et al.* (2011) recently showed that NCDase is a key participant of ceramide formation in liver mitochondria. The activity of NCDase causes formation of an additional source of ceramide in mitochondria by catalyzing a reverse reaction where formation of ceramide is the result of condensation of palmitate and SPH (Novgorodov *et al.* 2011). On the basis of molecular cloning and confocal microscopy data, this activity was ascribed to mitochondria (El Bawab *et al.* 2000); and it was demonstrated in purified mitochondria (Bionda *et al.* 2004). A recent report suggests that ceramide could be also generated by novel mitochondrial neutral SMase hydrolyzing SM (Wu *et al.* 2010). Given the existence and the likelihood for each one of these various pathways to occur in the mitochondria, continued research efforts are required to better understand the mechanisms of mitochondrial ceramide generation and utilization, along with their influence on mitochondrial functions.

Because of the importance of sphingolipids as components of membranes, it is possible to speculate that reduced sphingolipid levels in mitochondria may either increase the demand on, and/or decrease the capacity of mitochondrial function by affecting mitochondrial membrane dynamics. Increased load (of either handling or processing) of defective membrane organelles could exceed the capacity of oocyte mitochondrial respiration, resulting in cellular demise. Before this final outcome, decreases in mitochondrial function might manifest as slow changes in oocyte quality.

Summary

Thus, it seems increasingly likely that certain changes/shifts in the metabolism of ceramide in subcellular compartments of the oocyte are associated with signaling of biochemical or morphological alterations linked to aging. Nevertheless, the downstream targets of ceramide signaling or other yet undescribed early signals activated by aging in germ cells remain to be fully elucidated. Our data point toward the involvement of ceramide in the age-dependent changes in the structure and function of oocyte mitochondria. Although most studies are often centered on searching for a unitary function of ceramide, the ensuing observations often reveal not only several functions, but also at times contradictory ones depending on cell type and other variables. Therefore, this scenario renders a unified understanding of ceramide function difficult, if not misleading. For example, in contrast to previous reports suggesting that ceramide is proapoptotic *in vitro* and can mediate both stress-induced intrinsic and death receptor-mediated extrinsic apoptosis (Taha *et al.* 2006, Eliyahu *et al.* 2007, 2010, Lahiri & Futerman 2007), our data suggest that reduction of mitochondrial ceramide in oocytes depresses oocyte quality. Our data agree with studies where reduction of ceramide or complex sphingolipids results in progressive death of neurons, particularly Purkinje cell loss (Zhao *et al.* 2011). Moreover, intracerebroventricular administration of global ceramide inhibitors has been reported to cause acute neurodegeneration (Osuchowski *et al.* 2005). Therefore, contrary to the *in vitro* data, those findings, together with our results, demonstrate that decreases in ceramide synthesis can have devastating cellular consequences *in vivo*. Since under normal conditions sphingolipid homeostasis is critically balanced in the cell, it is not totally unexpected to find that either increases or decreases of ceramide are equally detrimental to the cell.

Alternatively, ceramide species possessing different fatty acyl chains may play distinct physiological roles. For example, loss of one of the two ceramide synthases in *Caenorhabditis elegans*, which produce different ceramide species, resulted in opposite outcomes under hypoxic conditions (Menuz *et al.* 2009). Thus, conceptualization of a unitary ceramide function is deceptive; but rather, a possible common theme is that individual molecular species of ceramide are likely regulated by specific biochemical pathways in distinct subcellular compartments, and modulate distinct functions. Therefore, endogenous ceramide production should be considered in its topological context. For example, we hypothesize that, in women, beyond a certain age, decreases of ceramide in the mitochondria, coupled with increases in the ER contribute to diminished oocyte quality.

As to what triggers these alterations, and what stoichiometric ratios are critical or are at the threshold remain unclear. But, we postulate that either transport or biosynthesis of ceramide is critical for organelle homeostasis. Thus, oocyte aging is precipitated by shifts in the synthesis and the catabolism of ceramide, in concert with changes in biochemical and structural properties of mitochondria.

This scenario reminds us that sphingolipid metabolism is highly connected and integrated. Therefore, attempts at dissecting specific pathways of ceramide metabolism and function need to consider not only ceramides but also their metabolites that may mediate their own specific actions. In any case, and despite the culprit (C16-ceramide, or CERT, or CerS6, or NCDase) responsible for decreases in oocyte quality with age, studying oocyte lipid biology opens new and exciting areas of research awaiting to be fully explored.

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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