2013 Research Grant Program Winning Abstract

Establishing Oogonial Stem Cells as a New Model to Treat Age-Associated Infertility

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The loss of stem cell function over time is considered a major cause of age-related organ deterioration and disease. In the context of regenerative medicine and curing age-related diseases, two main categories of stem cells have attracted much attention: adult somatic stem cells and embryonic stem cells. A third category, the germ cell, has received much less attention. Existence of stem cells in the human female germline was only recently demonstrated. Of all the major organs in the body, ovaries are the first organs to fail with advancing age, starting with reduced oocyte number and quality. Understanding how ovarian stem cells maintain their immortality and why over time they fail to generate new oocytes could reveal fundamental mechanisms of aging and cellular immortality.

One of the leading theories of oocyte aging is that mitochondrial function declines with age. Consistent with this, oocytes from old individuals have intracellular clusters of aggregated mitochondria that are increased in size and density. Since mitochondria in the offspring are solely inherited from the mother, age-related maternal mitochondrial dysfunction contributes to an increased risk of metabolic pathologies in the adult life of offspring.

Oocyte precursor stem cells have been recently isolated from mouse and human adult ovaries. These immortal germline stem cells, referred to as oogonial stem cells (OSCs), generate oocytes in culture and can actively replenish the pool of oocytes in young and adult mouse ovaries. In transplantation studies, mouse OSCs have been shown to differentiate into oocytes that mature, fertilize, and produce viable offspring. Since mitochondrial dysfunction is strongly implicated in aging, it is reasonable to propose that the development of a quiescent state in OSCs from old ovaries is a result of an age-linked mitochondrial defect. Thus, investigating age-dependent alterations in mitochondrial function of the OSCs could lead to insights into why stem cells age and why ovaries fail over time.

There is increasing evidence suggesting a decline in NAD+ levels underlying mitochondrial dysfunction in multiple diseases of aging, including aging itself. To test if mitochondrial decline contributes to the aging of OSCs, we have identified novel “mitochondrial enhancing molecules” (MEMs) that inhibit CD38, a ubiquitous enzyme that hydrolyzes NAD+. Consistent with predictions, inhibition of CD38 increases mitochondrial function and bioenergetics of OSCs in culture. Unpublished results from our lab demonstrate that the MEMs increase NAD+ levels and significantly improve mitochondrial function in OSCs.

In this proposal, we aim to test the hypothesis that dormancy of OSCs in old females is due to a decline in mitochondrial metabolism and ATP production, which can be restored

with agents that raise NAD+ levels. We propose the following specific aims:

Specific Aim 1: To use OSCs as a model to understand fundamental aging mechanisms. The OSCs will be isolated from young and old (3 vs 12 month old) female mice, and their physiology and mitochondrial function will be compared. We will also quantify the number of oocytes and evaluate oocyte quality by assessing mitochondrial staining pattern and spindle assembly. Confocal analysis of the oocyte spindles will be performed using antibodies from BD. A critical step in this aim is to isolate OSCs from mice. This will be done by dissociating ovaries into single cells and labeling them with germ cell marker Ddx4 and secondary antibodies from BD. The sorting of Ddx4+/+ cells will be performed using Falcon® tubes (Corning) and a BD FACSAria™ cell sorter. Once the OSCs are sorted, they will be cultured ex vivo. Cultured cells from young and old mice will be characterized extensively for their germline markers, cellular phenotype, and gene expression by immunofluorescence, RT-PCR, and flow cytometry using antibodies and reagents from BD. We will measure mitochondrial activity, ATP production, mtDNA copy number, and mitochondrial membrane potential in OSCs from young and old mice. Mitochondrial membrane potential will be measured by the BD mitochondrial membrane potential detection kit and analyzed using a BD FACSCalibur™ flow cytometer.

Specific Aim 2: To determine if raising NAD+ levels in vivo delays ovarian aging and extends functional lifespan of OSCs. We hypothesize that MEMs, by improving mitochondrial function, will reverse the metabolic defect in OSCs leading to increased fertility. We will feed young and old mice with diets constituting the MEMs. Isolation and quantification of OSCs from diet-fed mice will be done by flow cytometry using reagents from BD. Oocytes will be counted post-treatment and quality assessed by analyzing the meiotic spindle assembly using antibodies from BD for immunofluorescence. Finally, we will conduct mating trials to determine whether these compounds improve reproductive potential and extend reproductive lifespan of mice.

This proposal aims at identifying new pharmacological interventions to slow age-associated loss of fertility. If successful, this proposal will enable us to identify mechanisms underlying the declination of germ cell function during aging. The advantage of using OSCs is that they provide a platform to screen for compounds that by increasing NAD+ levels improve energetics and possibly oocyte quality. These proposed studies will make extensive use of flow cytometry and other reagents from BD. We also rely heavily on BD plasticware, including cell culture flasks, plates, conical tubes, pipets, and cell strainers.
